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Responses of cytochrome P4501A in freshwater fish exposed to bleached kraft mill effluent in experimental stream channels

Laura A. Bankey, Peter A. Van Veld, Dennis L. Borton, Larry LaFleur, and John J. Stegeman

Abstract: Cytochrome P4501A induction was used to evaluate exposure of fish to bleached kraft mill effluent in experimental stream channels. Immunodetectable P4501A and associated ethoxyresorufin-*O*-deethylase (EROD) activity were elevated in largemouth bass (*Micropterus salmoides*) and channel catfish (*Ictalurus punctatus*) throughout 263 days of exposure to 8% and 4% effluent from a mill that used 70% chlorine dioxide substitution for chlorine in the pulp bleaching process. In largemouth bass, P4501A was elevated as high as 55-fold over control fish. Large temporal and species variations were observed in enzyme levels and activity. Levels of chlorinated organic compounds present in effluent also varied throughout exposures. However, there was no apparent relationship between P4501A and any of those compounds measured. P4501A and EROD in channel catfish declined to control levels within seven days after transfer of fish to clean water or after terminating introduction of effluent into the channels. In another exposure, induction was observed in largemouth bass at 4 and 12% effluent after the mill began using oxygen in the delignification process and 100% substitution of chlorine dioxide for chlorine.

Résumé : Nous nous sommes servis de l'induction du cytochrome P4501A pour mesurer dans des chenaux expérimentaux l'exposition des poissons à l'effluent d'une usine de pâte kraft blanchie. La présence immunodétectable du P4501A et l'activité associée de l'éthoxyrésorufine-*O*-déséthylase (EROD) étaient élevées chez l'achigan à grande bouche (*Micropterus salmoides*) et chez la barbue de rivière (*Ictalurus punctatus*) au long des 263 jours d'exposition à 8 % et 4 % d'effluents d'une usine qui remplaçait 70 % du chlore par du dioxyde de chlore dans le procédé de blanchiment. Chez l'achigan à grande bouche, la présence du P4501A était jusqu'à 55 fois plus élevée que chez les poissons témoins. On a observé de fortes variations temporelles et spécifiques dans les concentrations et dans l'activité des enzymes. Les teneurs de l'effluent en composés organiques chlorés variaient aussi pendant l'exposition. Il ne semblait toutefois pas y avoir de relation entre le P4501A et les composés mesurés. Chez la barbue de rivière, le P4501A et l'EROD sont redescendus au niveau des poissons témoins en sept jours après le transfert des poissons vers des eaux propres ou après la fin de l'introduction de l'effluent dans les chenaux. Dans un autre expérience, on a observé l'induction chez l'achigan à grande bouche en présence de 4 % et 12 % d'effluent après que l'usine a commencé à employer l'oxygène dans le procédé de délignification et après un remplacement de 100 % du chlore par du dioxyde de chlore.

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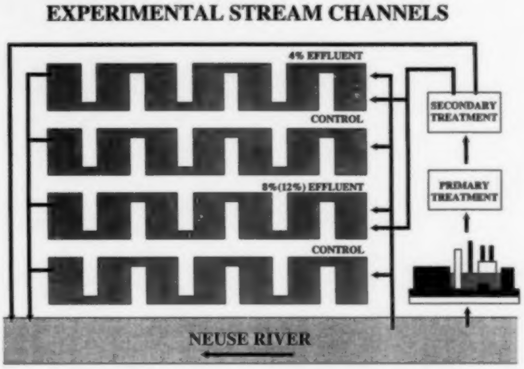
Introduction

Cytochrome P4501A proteins are members of a family of enzymes collectively known as cytochromes P450 that are central catalysts in the biotransformation of diverse xenobiotic and endogenous compounds (Black and Coon 1987; Stegeman 1989). A common feature of this family is induction of specific forms by exposure to various xenobiotics. P4501A forms are induced by exposure to specific polycyclic aromatic hydrocarbons and halogenated organic compounds such as polychlorinated biphenyls, polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). P4501A responds rapidly (e.g., hours) to environmental levels of inducers in a dose-dependent manner. Sustained induction is sometimes observed for extended periods following exposure (Hahn et al. 1989). Induction can be evaluated by immunodetection using highly specific antibodies to the enzyme or by catalytic measurement such as ethoxyresorufin-*O*-deethylase (EROD) activity (Stegeman et al. 1987). Evidence for elevated levels of the enzyme has been observed in diverse environments (Elskus and Stegeman 1989; Van Veld et al. 1990), including those receiving bleached kraft pulp mill effluent (BKME) (Oikari et al. 1985; Mather-Mihaich and Di Giulio 1991; McMaster et al. 1991; Munkittrick et al. 1991).

The agents in BKME responsible for induction have not been identified. Historically, elemental chlorine (Cl_2) has been used in pulp bleaching, resulting in the formation of chlorinated organic compounds in BKME (Oikari et al. 1985; McLeay 1987). While the contribution of organochlorines to P4501A induction and to the overall toxicity of BKME is not well understood (Owens 1991; Leach and Thakore 1977; O'Connor et al. 1993), the discovery of PCDDs and PCDFs in effluents has been a recent concern (Swanson et al. 1988; Fletcher and McKay 1993). The relatively high toxicity of these compounds observed in some species (Safe 1986; Poland and Knutson 1982) has prompted efforts to reduce or eliminate all chlorinated compounds from pulp and paper effluents. The recent use of chlorine dioxide (ClO_2) as a substitute for Cl_2 in pulp bleaching has been shown to substantially reduce the production of chlorinated organic compounds (Berry et al. 1989). More recently the use of oxygen in the delignification process prior to pulp bleaching has been shown to reduce the production of lignin-derived chlorinated organics (Berry et al. 1991). The combination of oxygen delignification and ClO_2 substitution is being implemented with increasing frequency.

P4501A induction is the most consistent and sensitive indicator of exposure to BKME known (Owens 1991) and may be useful in evaluating the effectiveness of changes in technology in reducing levels of toxicant exposure in aquatic organisms. In this study, we examined the responses of P4501A and EROD in two species of fish during long-term exposure to BKME in experimental stream channels. The first exposure was conducted when the mill adjacent to the channels was using 70% substitution of ClO_2 for Cl_2 in pulp bleaching. Temporal variations were examined and the relationship between induction and aqueous concentrations of selected organic compounds was explored in an attempt to identify potential inducers. Post-exposure experiments were conducted in order to better understand properties of the inducer(s). Long-term studies were also conducted after the mill had converted to oxygen delignification and 100% ClO_2 substitution to examine the effects of mill process changes on enzyme levels and activity.

Fig. 1. Schematic diagram of NCASI warmwater experimental stream channels.



Materials and methods

Experimental channels

The National Council for Air and Stream Improvement (NCASI) experimental stream channels are designed to approximate the complexity of natural ecosystems while being amenable to controlled exposure studies (NCASI 1983, 1989*a*). Being adjacent to an operating pulp and paper mill, the channels can receive measured dilutions of BKME over defined periods of time. Problems associated with migration of fish and with unequal and undefined BKME exposure histories are essentially eliminated.

The four warmwater stream channels are located near a pulp mill and adjacent to a freshwater section of the Neuse River in eastern North Carolina. Channels are 110 m long and consist of alternating arrangements of seven pools (1 m deep × 8 m long × 8 m wide) and six riffles (0.1 m deep × 8.6 m long × 2 m wide) with corresponding areas of slow and faster moving water (Fig. 1). For the present study, each stream channel was continuously supplied with Neuse River water at a rate of approximately 1020 $L \cdot min^{-1}$ pumped from an area upstream of the mill's effluent discharge site. This flow rate provided approximately 10 turnovers of stream water per day. Effluent was diverted from the mill and diluted to desired concentrations described below with the river water.

Stocking, exposures, post-exposures, and sampling

For the first experimental period, two streams served as controls (0% effluent), while the other two streams received either 4 or 8% ClO_2 effluent. After approximately 1 month, channels were stocked with immature hatchery-reared largemouth bass (*Micropterus salmoides*) and channel catfish (*Ictalurus punctatus*) obtained from the Cape Fear Fish Hatchery (Wilmington, North Carolina). Exposure began in October 1990 and ended in July 1991. Fish were sampled on days 0, 26, 54, 110, 172, and 262–265 (hereafter referred to as day 263). Largemouth bass were not sampled on day 110 or 172 because limited numbers were available.

Following sampling, some of the remaining fish were placed in 250-gallon tanks that received flowing well water for a 30-day post-exposure period. Fish were sampled on days 7 and 30 of post-exposure. Other channel catfish from the former 8% stream channel remained in that channel for a post-exposure during which the stream was supplied with Neuse River water only. The purpose of this experiment was to evaluate responses to possible contaminants that may have accumulated in sediments during the ClO_2 effluent exposure. Samples were collected on days 7 and 30.

At the end of the post-exposure experiment, streams were emptied and most of the sediments were removed. Streams were stocked with immature largemouth bass and subsequently received 0, 4, or 12% oxygen delignification/ClO_2 effluent. Bass were sampled after 170, 312, and 375 days of exposure. In another study, immature channel catfish were exposed to either 0, 4, or 12% oxygen delignification/ClO_2 effluent in 250-gallon tanks. Well water was used as control and dilution water. Flow volumes were sufficient for approximately four exchanges per day. Fish were sampled on day 30. Largemouth bass and channel catfish used in the oxygen delignification/ClO_2 exposures were also from the Cape Fear Fish Hatchery but were of a different stock than those used for ClO_2 effluent exposures.

On sampling days, fish were netted, anesthetized one at a time, weighed, and immediately immersed in ice-cold 1.15% KCl. Body cavities were opened, livers were removed, rinsed in ice-cold 1.15% KCl and frozen in liquid nitrogen. Upon arrival at the laboratory, livers were stored at −80°C until analysis.

For analysis of organic compounds, effluent samples were collected once weekly from a location at the head of the stream channels immediately before entry into the dilution chambers. Effluent was handled as described previously (NCASI 1986) prior to analysis.

Microsome preparation

All steps in the preparation of microsomes were performed at ice-cold temperatures according to procedures described previously (Van Veld et al. 1988). Briefly, livers were thawed and homogenized in buffer A consisting of 100 mM potassium phosphate (pH 7.4), 1 mM dithiothreitol, 1 mM EDTA, 0.1 mM phenylmethyl sulfonyl fluoride (PMSF), and 20% glycerol. The Polytron tissue homogenizer (Brinkman Instruments, Westbury, N.Y.) used in homogenization was equipped with a PTA 10TS generator designed to minimize frothing. Homogenates were centrifuged twice at 12 000 *g* for 10 min, the pellet being discarded after each centrifugation. The 12 000 *g* supernatant was then centrifuged at 100 000 *g* for 1 h. The resulting microsomal pellet was frozen in liquid nitrogen until analysis.

Microsomal protein, cytochrome P450, and EROD measurements

Total microsomal protein was determined by the method of Bradford (1976) using BSA as a standard. To assure stability of microsomal preparations, total spectral cytochrome P450 was evaluated by a carbon monoxide difference spectra method described by Stegeman et al. (1987). Ethoxyresorufin-*O*-deethylase (EROD) activity was measured spectrophotometrically (Klotz et al. 1984). EROD reaction mixtures consisted of 100 mM Tris–HCl (pH 8.0), 0.1 M NaCl, 2 μM 7-ethoxyresorufin (Molecular Probes, Inc., Eugene, Oregon), 0.40 mM NADPH, and microsomal protein (100 μg/mL). Blanks consisted of complete reaction mixtures minus NADPH.

Immunodetection of P4501A

Immunoblot analysis of P4501A was performed as described by Kloepper-Sams et al. (1987) with modifications. Microsomal proteins were separated on 12% polyacrylamide gels and transferred to 20 μm nitrocellulose paper. Monoclonal antibody 1-12-3 to scup P4501A (Park et al. 1986) was used as the primary antibody. The secondary antibody was alkaline phosphatase-conjugated goat anti-mouse IgG (Bio-Rad Inc., Richmond, Calif.). Staining was performed using *p*-nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indoyl phosphate (BCIP). Hepatic microsomal P4501A from spot (*Leiostomus xanthurus*) previously calibrated against purified scup P4501A (Klotz et al. 1983) was used to generate standard curves. The amount of stain was quantified with a thin-layer chromato scanner (Shimadzu, Kyoto, Japan).

Effluent chemistry

TCDD and TCDF congeners were quantified using methods previously described (NCASI 1989*b*). Aliquots of effluent received internal standards [$^{13}C_{12}$-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and $^{13}C_{12}$-2,3,7,8-tetrachlorodibenzofuran (TCDF)] prior to being filtered through glass fiber filters. The glass fiber filters were soxhlet extracted with 68:32 (v/v) ethanol/toluene. Ethanol was added to the filtrates that were then adjusted to pH 1 prior to extraction with methylene chloride. Samples then received $^{37}Cl_4$-1,2,3,4-TCDF as an internal standard for the cleanup procedure. For cleanup, extracts were treated with an acid/base wash, dried, and subjected to column chromatography involving NaOH-silica:silica:H_2SO_4-silica/acid alumina columns followed by charcoal/silica gel columns. All samples then received $^{13}C_{12}$-1,2,3,4-TCDD and were injected into a high resolution gas chromatograph/high resolution mass spectrometer (HRGC/HRMS) in the electron ionization mode for selective ion monitoring. Compounds were identified by comparison of the ratio of the integrated abundance of the M^+ and $M + 2^+$ ions to their theoretical abundance ratio and peak signal-to-noise ratio of >3.0:1. Chlorinated phenolics were quantified as described previously (NCASI 1986).

Data analysis

Sample variance for P4501A and EROD during the effluent exposure was determined to be nonhomogeneous. Data was therefore analyzed by Kruskal–Wallis analysis of variance followed by the Bonferroni test of multiple comparisons to identify differences ($p = 0.05$) in P4501A responses between treatments. Significant differences ($p = 0.05$) between treatments in the oxygen delignification experiments were determined by analysis of variance.

Results

Responses to ClO_2 effluent

Carbon monoxide difference spectra of hepatic microsomes throughout the entire study gave results typical of stable

Fig. 2. Representative immunoblots of liver microsomes from fish exposed to ClO$_2$ effluent and oxygen delignification/ClO$_2$ effluent. Lane 1, control channel catfish; lane 2, channel catfish, 8% ClO$_2$ effluent; lane 3, control largemouth bass; lane 4, largemouth bass, 8% ClO$_2$ effluent; lane 5, largemouth bass, 12% oxygen delignification/ClO$_2$ effluent; lanes 6–9, microsomal P4501A standards.

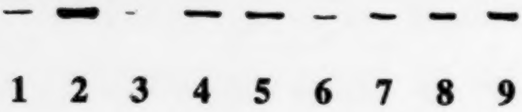


Fig. 3. Temporal variation in hepatic P4501A and EROD in largemouth bass exposed for 263 days to ClO$_2$ effluent. Data points represent the means ± 1 SD (n = 4 or 5 for samples collected on days 0, 26, and 54; n = 12 for samples collected on day 263).■, 8% effluent; ●, 4% effluent; ▲, control.

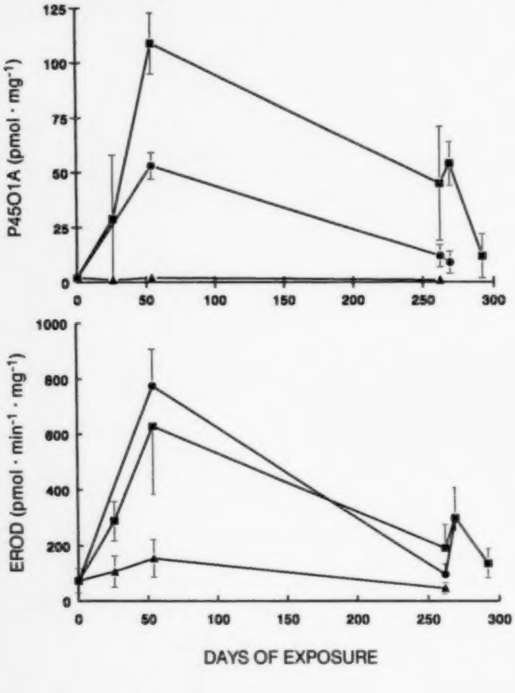
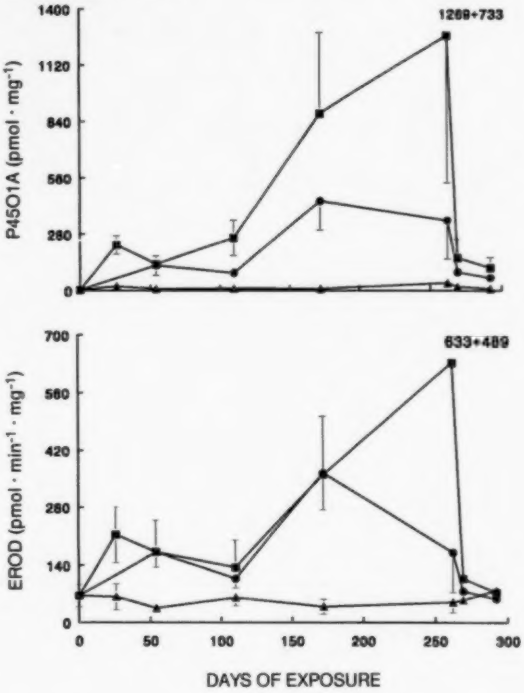


Fig. 4. Temporal variation in hepatic P4501A and EROD in channel catfish exposed for 263 days to ClO$_2$ effluent. Data points represent the means ± 1 SD (n = 4 or 5 for samples collected on days 0, 26, 54, 110, and 172; n = 12 for samples collected on day 263). ■, 8% effluent; ●, 4% effluent; ▲, control.



P450 with little or no indication of degradation of P450 to its inactive form (P420) (data not shown). The microsomal samples were therefore judged suitable for measurements of catalytic (EROD) activity.

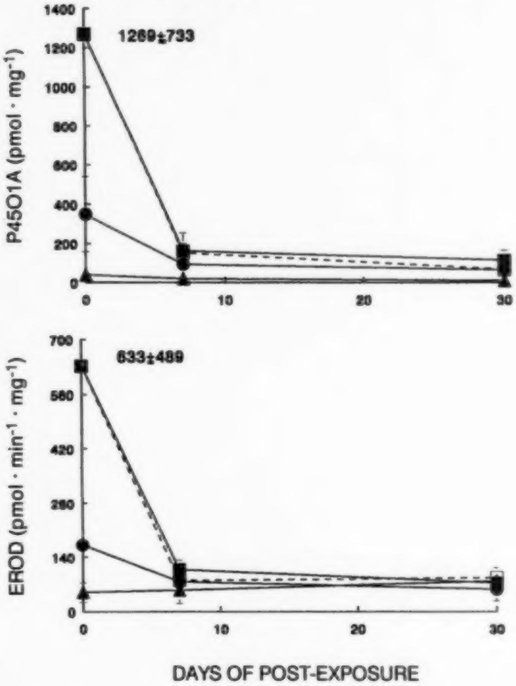
Monoclonal antibody 1-12-3 to P4501A recognized a single protein band (molecular mass approximately 54 kDa) in hepatic microsomes from largemouth bass and channel catfish as typically seen in other teleosts (Stegeman 1989) (Fig. 2). Levels of P4501A and associated EROD activity were consistently higher in exposed fish than in fish collected from control streams (Figs. 2–4). Immunodetectable P4501A (with the exception of bass P4501A, day 54) was consistently higher in fish collected from 8% effluent compared with those from 4% effluent.

Large temporal variations in the range of values for P4501A and EROD were observed in exposed fish but were not observed in control fish (Figs. 3 and 4). Patterns of temporal variation were different between species. For example, the highest largemouth bass P4501A and EROD were observed on day 54 in both 4 and 8% streams, while the lowest values were observed at the end of exposure (Fig. 3). In largemouth bass from the 8% channel on day 54, P4501A and EROD exceeded that of control fish by approximately 55- and 4-fold, respectively. In contrast with the results with largemouth bass, channel catfish exhibited relatively high P4501A and EROD at the end of exposure (Fig. 4). On day 263, in channel catfish from the 8% channel, P4501A and EROD exceeded controls by approximately 33- and 13-fold, respectively.

Post-exposure experiments

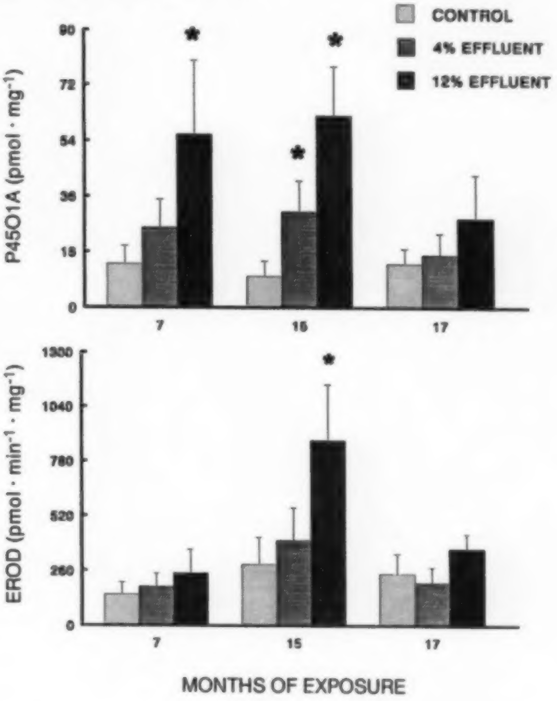
Following the ClO$_2$ effluent exposure, groups of channel catfish were placed in clean well water systems, as described above, for a 30-day post-exposure period. On days 7 and 30 of post-exposure, there were no significant differences in the levels of P4501A and EROD in previously exposed versus control channel catfish (Fig. 5). In another

Fig. 5. Catfish liver P4501A and EROD during post-exposure experiments. Fish were placed in well water systems after previous exposure to either 8% effluent (■) or 4% effluent (●). Some fish remained in former 8% streams (8% sediment, □---□), while the streams received river water only. Values represent the mean ± 1 SD (n = 12 for samples collected at time 0 and n = 5 for all other samples). ▲, control.



post-exposure experiment, channel catfish in the former 8% channel were maintained in that channel for an additional 30 days, while it was supplied with Neuse River water only. Enzyme levels and activity declined after 7 days and were insignificant from controls after 30 days (Fig. 5).

Fig. 6. Largemouth bass P4501A and EROD in fish exposed for 11 months to oxygen delignification/ClO$_2$ effluent. Bars represent means ± 1 SD (n = 7 for samples collected from 0 and 12% effluent and n = 5 for fish collected from 4% effluent).



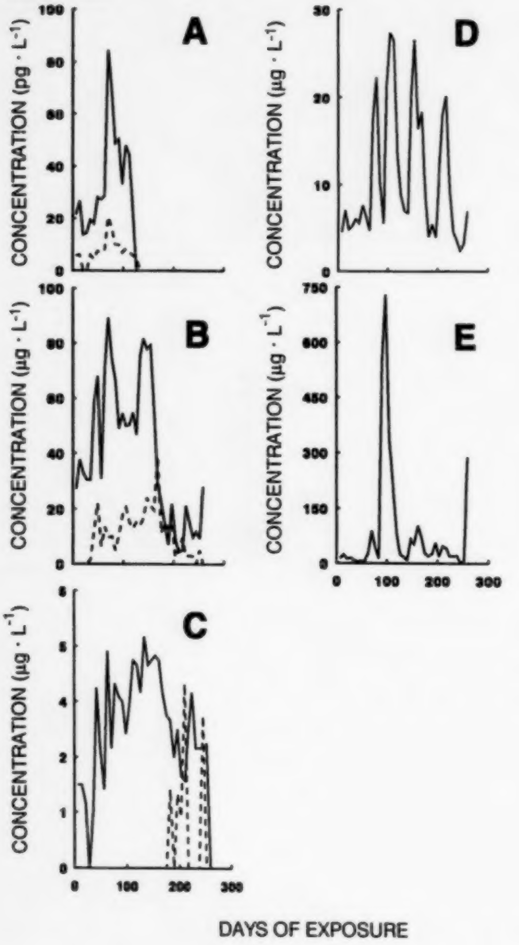
Responses to oxygen delignification/ClO$_2$ effluent

P4501A and EROD were evaluated in largemouth bass during 375 days of exposure to oxygen delignification/ClO$_2$ effluent (Fig. 6). P4501A in fish collected from 12% effluent was significantly elevated relative to control fish on two of three sampling dates. Highest (sixfold) induction was observed in fish collected on day 312 from the 12% effluent. P4501A was also significantly elevated (threefold) in fish collected from 4% effluent on day 312. There was a trend towards higher EROD activity in exposed fish; however, significant EROD elevation (threefold) was observed only in fish from 12% effluent on day 312. In another experiment, channel catfish were exposed to 12% oxygen delignification/ClO$_2$ effluent in 250-gallon flow-through tanks. Well water was used for dilution and controls. Following 30 days of exposure, there was a trend for higher EROD in exposed fish (152 ± 52 pmol·min^{-1}·mg^{-1}; n = 7) compared with controls (72 ± 40 pmol·min^{-1}·mg^{-1}); however, the difference was not significant. Overall, the fold-induction observed in fish exposed to oxygen delignification/ClO$_2$ effluent appeared less than that observed in fish exposed to ClO$_2$ effluent described above.

Effluent chemistry during the ClO$_2$ effluent exposure

The concentration of 2,3,7,8-TCDD found in the effluent was below 6 pg/L for the first 40 days of the exposure, reached a high of 20 pg/L near day 70 of the exposure and gradually declined to levels of less than 5 pg/L during the last 120 days of exposure. The concentration of 2,3,7,8-TCDF followed a similar pattern but was usually about four times greater than the concentration of 2,3,7,8-TCDD and reached a high of 85 pg/L near day 70 (Fig. 7A). The total of nine chlorocatechols ranged from nondetectable (detection limits ≤2 μg/L) to 34 μg/L, while the total of three chlorovanillins (primarily 6-chlorovanillin) ranged from 4 to 89 μg/L over the exposure (Fig. 7B). The total concentration of seven chlorophenols ranged from nondetectable to 4 μg/L and were greatest approximately midway through the experiment (Fig. 7C). 2,4-dichloro- and 2,4,6-trichlorophenol were usually the only chlorophenols detected in the effluent. The total of 10 chloroguiacols ranged from nondetectable to 6 μg/L over the exposure (Fig. 7C). The

Fig. 7. Concentrations of selected organic compounds during the ClO_2 effluent exposure. (A) 2,3,7,8-TCDF (solid line) and 2,3,7,8-TCDD (dashed line). (B) Total of three chlorovanillans (solid line) and nine chlorocatechols (dashed line). (C) Total of chloroguiacols (solid line) and seven chlorophenols (dashed line). (D) Total of three chlorinated resin acids. (E) Total of 10 nonchlorinated resin acids.



total of three chlorinated resin acids ranged from 2 to 30 µg/L and appeared in several pulses over the exposures (Fig. 7D). The total of 10 resin acids ranged from lows near 3 µg/L early and late in the exposure to a high of 762 µg/L on one occasion near day 100 (Fig. 7E). Abietic and dehydroabietic acid were usually accounted for 80% or more of the resin acids measured.

Effluent chemistry during the oxygen delignification/ClO_2

Effluent exposure

During the exposure to the oxygen delignification/ClO_2 effluent the amounts of chlorinated organic compounds found in the effluent were greatly reduced (data not shown). Detectable levels (>5 pg/L) of 2,3,7,8-TCDD were found in only 5% of all samples and did not exceed 15 pg/L. Detectable concentrations of 2,3,7,8-TCDF were found in 47% of the samples and reached 73 pg/L on one occasion. With the exception of 6-chlorovanallin, which was found in detectable amounts up to 4 µg/L in 15% of the samples, all of the 29 chlorophenols, chloroguiacols, chlorocatechols, and chlorovanillins were less than the 2 µg/L detection limit in all 79 samples. Chlorinated resin acids were detected in only 12% of the samples and the highest concentration was 3 µg/L. The greatest concentration of the 10 resin acids measured was 418 µg/L, but the overall average was 79 µg/L, which was very similar to the 83 µg/L average found during the exposure to the ClO_2 effluent.

Discussion

In the present study, we demonstrate sustained induction of hepatic P4501A and associated EROD activity in fish during long-term exposure to environmentally relevant concentrations of BKME in experimental stream channels. Numerous studies have reported evidence for elevated P4501A-associated catalytic activities (e.g., EROD) in fish exposed to pulp mill effluents (Owens 1991). Many of these studies involved caging of fish at various distances from effluent discharge sites for defined periods (Lindström-Seppä and Oikari 1990; Oikari et al. 1985). This approach is effective in minimizing fish movement and has provided much useful information including information on temporal and spacial factors influencing BKME exposure and effects in the environment. However, aquatic organisms can accumulate toxicants during exposure to contaminated water, food, and sediment (Spacie and Hamelink 1985). Although the relative importance of these components in overall exposure is not understood, caging may alter exposure by altering normal feeding behavior, and contact with sediments. The stream channels used in our study may further contribute to our understanding by offering the advantage of controlled exposure, while allowing organisms to feed and behave in a manner that is similar to their behavior in natural waters.

During the course of the ClO_2 effluent exposure, large temporal variations in levels and activity of P4501A were observed in exposed fish. Such temporal variations are often related to the sex or changes in the reproductive status of mature fish (Larsen et al. 1992; Edwards et al. 1988). The fish used in the present study were sexually immature throughout the exposure period. This, in addition to the narrow range of temporal response in the control fish indicates that factors other than physiological changes related to sex and seasonal reproduction were responsible for the observed variations.

Levels of organic and chlorinated organic contaminants in the effluent varied during the experiment suggesting that fluctuations in one or more of these may have contributed to the temporal variation in enzyme levels. However, the species differences in temporal patterns of induction make it difficult to evaluate the possible involvement of the compounds selected for measurement. For example, effluent concentrations of PCDDs and PCDFs were highest during the first half of the ClO_2 exposure and declined to below detection levels during the second half. That largemouth bass enzyme followed a similar trend suggests that water-borne PCDDs and PCDFs may have contributed to the induction in this species. However, a different temporal pattern was observed in channel catfish suggesting that compounds other than aqueous PCDDs and PCDFs may be responsible for induction. Also, maximum concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF in fish during exposure never exceeded 2.5 and 5.0 pg/g, respectively (NCASI 1994). These values are below doses required to yield similar-fold inductions in other fish (van der Weiden et al. 1990; van der Weiden et al. 1993). The factors responsible for species differences in the present study are unknown but may be related to differences in foraging behavior, proximity to sediment-associated contaminants or other unidentified physiological and environmental factors.

Information on the nature of the agents responsible for induction in the stream channels was provided in the post-exposure experiments. The rapid decline in channel catfish enzyme levels during maintenance in clean systems would not be expected if the agents responsible were persistent lipophilic compounds such as PCDDs or PCDFs. Sustained induction of hepatic P4501A and associated activities are often reported weeks or months after exposure to PCDDs and PCDFs in a variety of species (Hahn et al. 1989; Muir et al. 1990; Muir et al. 1992; van der Weiden et al. 1993). The rapid decline in both P4501A and EROD in the present study is similar to results reported recently by other investigators (Munkittrick et al. 1992) who reported a rapid decrease in hepatic EROD activity in feral long-nose sucker shortly (days) after a temporary mill shutdown. Following exposure, the length of time required for induced enzyme levels to return to control or basal levels is likely to depend on a number of factors including the species being studied, the susceptibility of the inducer to biotransformation and elimination and the turnover rate of the specific enzyme and cells involved. While the influence of these factors was not determined here, our results suggest an inducer other than a persistent compound.

Upon entering aquatic ecosystems, PCDDs, PCDFs, and other lipophilic organic contaminants tend to associate with organic-rich sediments (Fletcher and McKay 1993; van der Weiden et al. 1989, 1993). That the predominant inducing agents were compounds other than persistent, lipophilic compounds was also suggested by our post-exposure experiment involving maintenance of previously exposed channel catfish in channels whose sediments had been previously exposed. In our study, the rapid decline of enzyme (i.e., within seven days) suggests either that the agents responsible for induction do not associate with sediment or that they were not available at sufficient levels to induce a response over the time allowed.

The contribution of PCDDs, PCDFs, and other chlorinated compounds to P4501A induction and other BKME-related effects remains an important and controversial issue (Owens 1991). Previous studies (Lehtinen 1990; Lehtinen et al. 1990) indicated that reduction in the levels of chlorinated compounds in BKME resulted in reduced biological effects including reduced EROD induction. Other investigators (Lindström-Seppä et al. 1992; Pesonen and Anderson 1992; Martel et al. 1994; Munkittrick et al. 1994) reported EROD induction in fish exposed to nonchlorinated effluents. We observed a general lower-fold elevation of P4501A and EROD in fish exposed to the oxygen delignification effluent with lower amounts of chlorinated organic compounds relative to those exposed to ClO_2 effluent. It must be borne in mind, however, that different mill technologies (e.g., oxygen delignification) may affect not only the levels and composition of chlorinated organic compounds but also affect the levels and composition of unchlorinated compounds as well.

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Prey field and diet of larval cod on Western Bank, Scotian Shelf

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Abstract: In vertically integrated samples (from ≤75 m) taken around and over Western Bank in November and December 1991, and January 1992, densities of larval cod (*Gadus morhua*) correlated with those of copepods, notably with relative densities of copepodids plus adults of predominant prey, *Pseudocalanus* spp. and *Paracalanus parvus*. *Pseudocalanus* showed a large increase from time of cod spawning but was concentrated over the bank through gyral circulation rather than enhanced productivity. Numbers of both copepods in stomachs of larvae <10 mm were significantly related to their field densities, when controlled for sample month, larva size, and time of day. Stomach fullness was related to sample month, time of day, size of larva, and density of *Pseudocalanus*, but not *P. parvus*. Smaller larvae from four depths at four stations over the bank in late 1992, ate mostly nauplii of *Pseudocalanus* and *Centropages typicus*. Numbers eaten were strongly related to their field densities, weakly to sample depth, and not to larva size. Stomach fullness was positively related only to densities of *Pseudocalanus* nauplii. Larvae >5–6 mm switched to larger prey, mostly *Pseudocalanus* copepodids. More research is needed on interactions between copepod behaviour and the physical mechanisms that concentrate or disperse them on offshore banks.

Résumé : Dans des échantillons verticalement intégrés (prélevés à ≤75 m) recueillis sur le banc Occidental et à ses abords en novembre et décembre 1991 et en janvier 1992, les densités de larves de morue (*Gadus morhua*) étaient corrélées avec celles des copépodes, notamment avec les densités relatives des copépodites et des adultes des proies prédominantes, les *Pseudocalanus* spp. et le *Paracalanus parvus*. On observait chez les *Pseudocalanus* une forte augmentation par rapport au moment de la ponte de la morue, mais ces organismes étaient concentrés au-dessus du banc sous l'action d'une circulation giratoire plutôt que d'un accroissement de la productivité. Les effectifs de ces deux types de copépodes dans les estomacs de larves <10 mm étaient liés de façon significative à leurs densités sur le terrain, lorsqu'on contrôlait le mois d'échantillonnage, la taille des larves et l'heure du jour. Le degré de remplissage des estomacs était lié au mois d'échantillonnage, à l'heure du jour, à la taille des larves et à la densité des *Pseudocalanus*, mais non de *P. parvus*. Des larves plus petites prélevées à quatre profondeurs et à quatre stations au-dessus du banc à la fin de 1992 consommaient essentiellement des nauplius de *Pseudocalanus* et de *Centropages typicus*. La quantité ingérée était fortement liée aux densités sur le terrain, faiblement liée à la profondeur d'échantillonnage et pas du tout liée à la taille des larves. Le degré de remplissage des estomacs n'était positivement lié qu'aux densités des nauplius de *Pseudocalanus*. Les larves de >5–6 mm passaient à des proies de plus grande taille, principalement aux copépodites de *Pseudocalanus*. Il est nécessaire de poursuivre la recherche sur les interactions entre le comportement des copépodes et les mécanismes physiques qui les concentrent ou les dispersent sur les bancs du large.

[Traduit par la Rédaction]

Introduction

The purpose of this study is to detail the dependence of larval cod on high concentrations of specific prey in an apparent offshore "nursery" area on Western Bank, Scotian Shelf, and to seek insights on the origin of this concentration. Such studies should help focus the search for biological and physical variables that may aid in predicting recruitment to important fisheries.

There has been much discussion, but little adequate field testing, of hypotheses relating recruitment of marine fishes to food for their larvae (see reviews by May 1974; Cushing 1975; Leggett 1986; Runge 1988; Cushing 1990; Poulet and Williams 1991). Only Ellertsen et al. (1989) have detailed an apparent match–mismatch relationship of cod larvae with the dynamics of identified prey, while others have been content to examine general seasonal, and sometimes spatial, concordance of peaks in occurrence of fish larvae with variously assessed concentrations of

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zooplankton (e.g., Bollens et al. 1992; Brander and Hurley 1992; Sherman et al. 1984; Skreslet 1989). Furthermore, a major problem stressed by Frank (1988) has been the inadequacy of the coarse-mesh nets used in ichthyoplankton surveys to catch items actually eaten by larvae. This caveat is noted by Brander and Hurley (1992) in their conclusion "that it is premature to reject the match hypothesis" for cod spawning on the Scotian Shelf. The few attempts to relate stomach contents to adequately sampled zooplankton have either been of limited temporal and spatial scope (e.g., Ellertsen et al. 1989) or have merely demonstrated feeding selectivity (e.g., Kane 1984). No field studies of larval cod have controlled for other pertinent variables to test hypotheses that amounts of prey consumed are related to densities of prey.

In recent years there has been some shift of focus onto hypotheses relating fish recruitment to horizontal transport, oceanographic fronts, and gyres (e.g., Sinclair 1988; Campana et al. 1989; Taggart et al. 1988). The area of larval cod concentration on Western Bank, for example, has been demonstrated to be a "quiet zone," within a persistent or recurrent gyre system (Bowen et al. 1995; K.R. Thompson, Department of Oceanography, Dalhousie University, Halifax, N.S., personal communication). Although retention in such "nursery" areas is itself considered by some to be important in maintaining appropriate behaviour of local stocks, such areas must also offer suitable feeding and predation regimes for larvae.

As part of the cod recruitment program of the Ocean Production Enhancement Network (OPEN), samples of plankton and fish larvae were collected roughly monthly over a 2-year period from a 45-station grid on the Scotian Shelf southwest of Sable Island (Fig. 1). Other dedicated cruises focused more closely within the OPEN grid on concentrations of larval cod or on the biology and physical oceanography of Western Bank, at the western extremity of Sable Island Bank (Fig. 1).

This study concerns cod larvae on Western Bank during the season of peak occurrence in 2 successive years: in late autumn through early winter 1991–1992 and during late November and early December 1992. Although concentrated spawning had been anticipated to occur within the OPEN grid, its greater prominence in late autumn than in spring was unexpected. Earlier ichthyoplankton surveys over a wider area of the Scotian Shelf concluded that most cod spawning took place during spring over a wide area, with a "more restricted" late autumn spawning centred west of Sable Island (Brander and Hurley 1992; see especially their Fig. 6). However, during the OPEN surveys of 1990–1993, spring spawning was much less evident (Frank et al. 1995; M.G. Meekan, Department of Biology, Laval University, Ste-Foy, Quebec, personal communication, Miller et al. 1995).

The monthly OPEN cruises were designed to delimit spawning events in time and space, and to assess morphometrics, lipid contents, birthdate frequencies and genetic characteristics of fish larvae (M.G. Meekan, personal communication, Miller et al. 1995). Although the vertically integrated plankton collections from the monthly cruises might not seem ideal for the purpose, we will show that a prey–predator relationship between larvae and zooplankton is nevertheless evident. Furthermore, these samples supply important insights into the biological oceanography of this major cod spawning area.

More detailed analyses of feeding of larval cod are based on a larval-tracking cruise in late 1992. The purpose of that cruise was to follow changes in the biological characteristics, including stomach contents, of individuals in a patch of cod larvae on Western Bank (S.E. Lochmann, Department of Oceanography, Dalhousie University, Halifax, N.S., personal communication). In addition, quantitative plankton samples were collected in fine-mesh nets from four depths at four stations to compare with stomach contents from cod larvae taken at these same stations and depths.

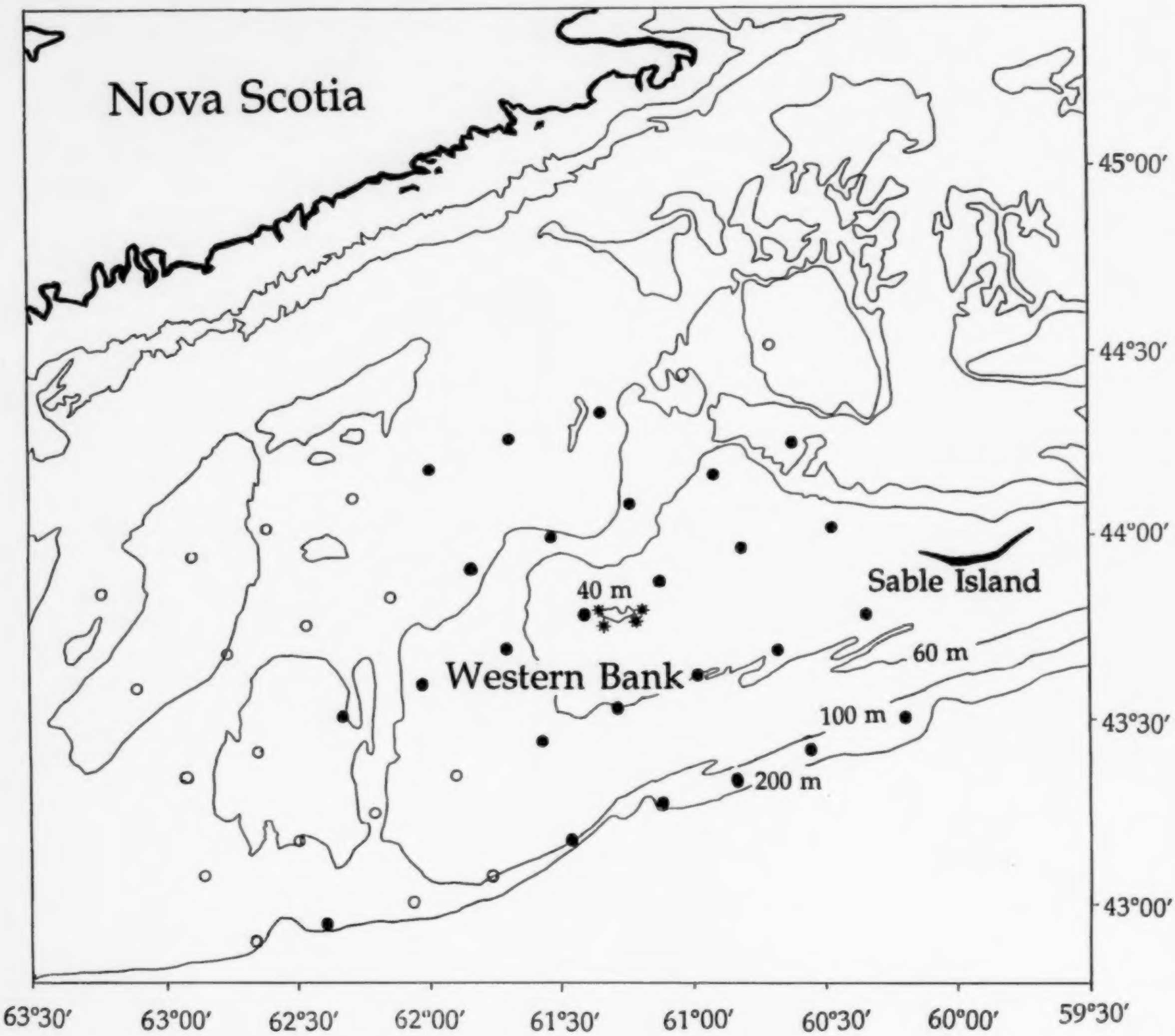
Materials and methods

Field collections

Plankton sampling over 2 years from the 45-station OPEN grid (Fig. 1) was intended for a variety of purposes. The samples used here were chosen and analyzed only within the context of the feeding of larval cod and were taken between 14–16 November and 10–12 December 1991, and 7–10 January 1992, from stations in the vicinity of Western Bank (Fig. 2). All plankton samples collected during the 3 months at stations falling within the area mapped on Fig. 2 were used. Because of program and weather contingencies, not all stations within the chosen area were sampled for plankton on each cruise. Cod larvae were sought by other gear unaccompanied by fine-mesh nets at some stations immediately west of Western Bank but were either absent or very rare (Fig. 2; M.G. Meekan, personal communication). Altogether plankton samples from 17 stations in November and December, and 20 in January, were available for the present study. Additional samples (1 in November, 2 in December, 6 in January) of larvae were taken over a 24-h period at a central station on Western Bank (closest to the 40-m contour "peak" on Fig. 1). A few plankton samples were also used to obtain information on zooplankton abundances over Western Bank from stations occupied between 13–16 September and 8–13 October 1991, before the seasonal appearance of cod larvae.

The zooplankton and larval fishes collected during the monthly OPEN surveys were obtained with a variety of nets. The zooplankton samples analysed here were obtained at random times through the 24-h day by a rectangular midwater trawl (RMT; Clarke 1969). Larger zooplankters and fish larvae were sampled by a metered RMT net, 2.83 × 4.0 m with a 333-μm mesh. A small, unmetered net, 15 cm diameter with a 64-μm mesh, was attached to the top of the RMT frame. The nets were deployed open and the RMT was lowered slowly to a maximum depth of 75 m, or 15 m from bottom where shallower, then raised slowly to the surface. Speeds were close to 2 knots, for a total towing time of 15–20 min, in a broad circle around the chosen station, which produced filtered volumes by the RMT in the order of 1000 m^3. In a few instances, flowmeter failures required interpolation of volumes from regressions of volumes filtered on distances towed. Parallel samples from the fine-mesh net included smaller copepodids, nauplii, and eggs, and were standardized as indicated below.

Fig. 1. Positions of stations in the OPEN grid on and around Western Bank, Scotian Shelf. All available plankton samples (at stations with solid symbols) obtained in November and December 1991, and January 1992, were used in the present analyses. Larvae were sought by gear other than that associated with plankton samples at some other stations but were not detected in shipboard samples (see Fig. 2). Positions of four stations sampled for plankton during a larval tracking cruise in late 1992 are shown with an asterisk.

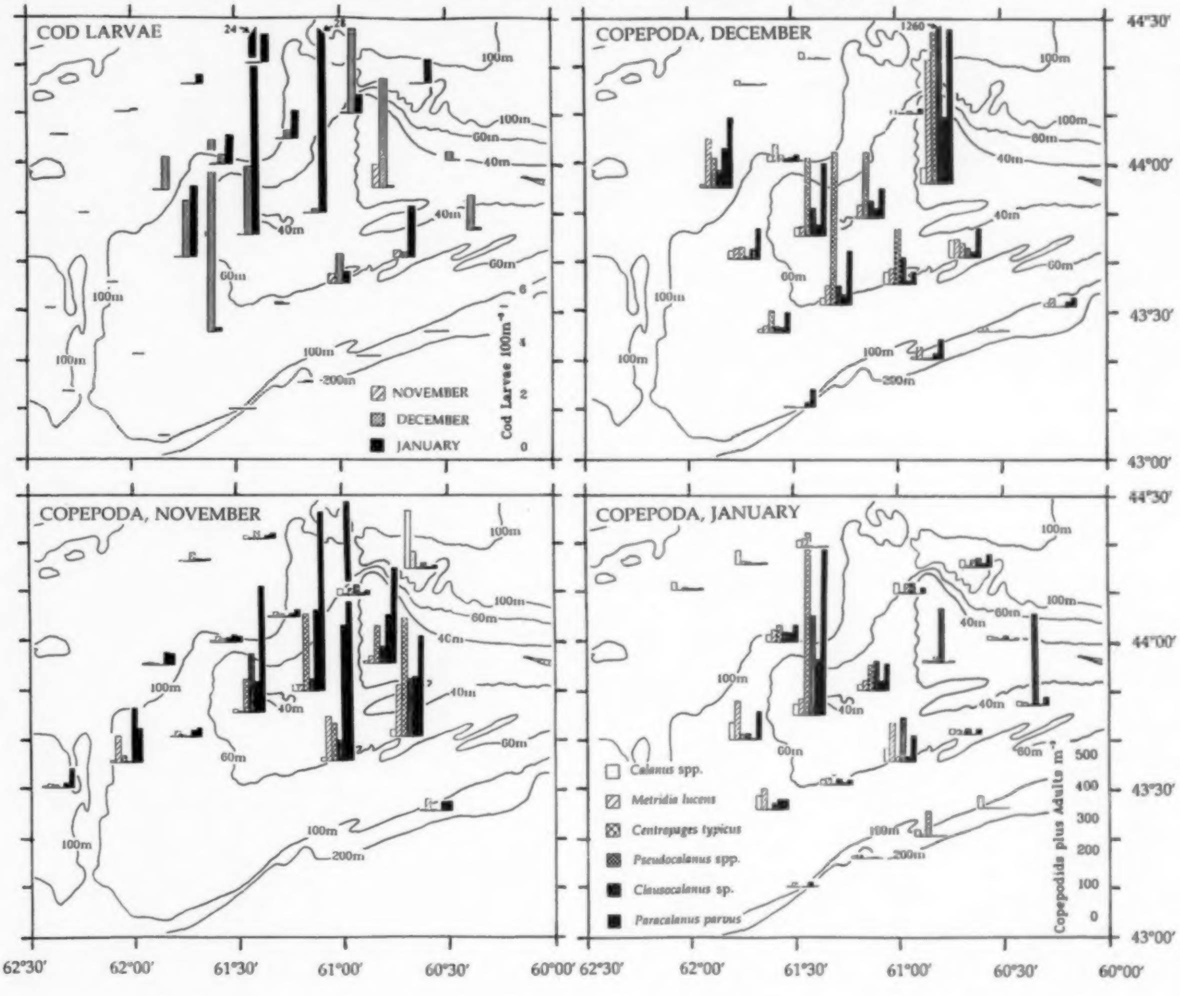


The coarse-mesh samples were searched aboard ship for fish larvae that were measured then stored in liquid nitrogen. Inevitably some were missed, so the shipboard counts (m^{-3}) of larval cod shown here reflect their *relative* abundances at the various stations; these relative abundances, with no known biases, are used only in rank analyses, and thus are adequate for present purposes. Definitive estimates are given elsewhere (M.G. Meekan, personal communication.)

Both fine-mesh and coarse-mesh samples were subdivided by plankton splitters aboard ship, or subsequently, and distributed among the various laboratories involved in OPEN. Samples available for our analyses consisted of 25 or 50% of the original samples (with fish larvae removed).

A quite different set of samples was collected during the larval-tracking cruise in late November and early December 1992. Cod larvae for analysis of stomach samples were collected from four stations over Western Bank (shown on Fig. 1). These larvae were taken from near the bottom to the surface by a series of BIONESS nets (Sameoto et al. 1980), 1 m^2, with a 333-μm mesh, towed obliquely at ca. 2 knots through 5-m strata. (Larval abundances are not used here.) Also at these stations, horizontal, quantitative,

Fig. 2. Abundances of cod larvae and predominant calanoid compepods (copepodids plus adults) at stations on and around Western Bank in late fall and early winter 1991–1992. The scales for Copepoda in November and December are the same as for January. No larvae were detected by shipboard sortings of samples from some stations (indicated by baseline dashes on the larval cod panel) that were not sampled for plankton. One station immediately beyond the southwest corner of the panels (see Fig. 1) was sampled for both larvae and plankton in November and is included in subsequent analyses.


COD LARVAE
▨ NOVEMBER
▩ DECEMBER
■ JANUARY
Cod Larvae 100m⁻³
0
2
4
6
24
28
COPEPODA, DECEMBER
1260
COPEPODA, NOVEMBER
COPEPODA, JANUARY
☐ Calanus spp.
▨ Metridia lucens
▩ Centropages typicus
▩ Pseudocalanus spp.
■ Clausocalanus sp.
■ Paracalanus parvus
Copepodids plus Adults m⁻³
0
100
200
300
400
500
40m
60m
100m
200m
44°30′
44°00′
43°30′
43°00′
62°30′
62°00′
61°30′
61°00′
60°30′
60°00′

fine-mesh plankton samples (Clarke-Bumpus sampler, 64-μm mesh) were collected at 5, 15, 25, and 35 m. The sampler was towed at each depth for ca. 5 min, giving sample volumes of ca. 3–5 m^3. All samples were collected in the afternoon, immediately before or after the associated BIONESS samples. Sampler failures and bottle breakage reduced these to 12 useable plankton samples that were associated with larvae from the same depth strata.

Laboratory analyses of plankton

From the monthly survey collections, macroplankters and larger meroplankters (which were not eaten by larvae) were identified and removed from the coarse-net samples but are not considered here. Late copepodids (CIV, CV) and adult *Calanus finmarchicus* and, when necessary (see below), adults of other large copepods were identified and counted in plankton-splitter subsamples as a basis for quantitative estimates of smaller copepods in the fine-mesh samples. This was done by systematic coverage of each subsample in a gridded Petri dish under a dissecting microscope using transmitted light.

Plankton-splitter subsamples of the fine-mesh samples from the monthly surveys were used for identification, counts, and measurements of smaller copepods. Calanoid adults and copepodids were identified and counted by systematic coverage of entire subsamples in gridded Petri dishes. Numbers of eggs and nauplii, and copepodids and adults of smaller zooplankton, including the abundant cyclopoid *Oithona similis* and harpactacoid *Microsetella norvegica* were estimated by further subsampling. These were counted at higher magnification in 10 microscopic fields (totalling ca. 1/15 of the subsample volume) placed evenly across Petri dishes containing well mixed subsamples. The estimated water-column densities of these smaller stages and taxa are accordingly less accurate than those for the calanoid copepodids and adults, although presumably unbiased.

It was clear from comparisons of numbers of zooplankters collected by the metered RMT nets and the associated small, unmetered fine-mesh net that the latter did not filter all the water encountered during a tow. There is a considerable and controversial literature on the avoidance of small nets by zooplankters. Avoidance by macroplankters is incontrovertible, but avoidance of rapidly towed nets by smaller, essentially sightless copepods is less serious (e.g., Fleminger and Clutter 1965). Also, a careful study by Nichols and Thompson (1981) indicates that virtually all copepods were retained by mesh sizes that were <75% of the prosome width of a particular stage or species. The RMT nets with mesh of 333 μm used in the OPEN surveys should thus have retained all copepodids and adults with prosome diameters exceeding ca. 450 μm; i.e., as large as or larger than adult *Centropages typicus*. However, there remains the possibility that the larger stages of *C. finmarchicus* were better able to avoid the small, fine-mesh net (but not the large RMT nets designed for quantitative sampling of fish larvae). We therefore hypothesized that there was no difference in the proportions of large *C. finmarchicus* (CIV to adult) versus the smaller adult *C. typicus* in the parallel tows of the fine and coarse nets used in the monthly surveys. This null hypothesis implies that both nets were similar in degree of avoidance by and retention of both taxa. We used all available samples (4 from November, 3 from December, and 1 from January), in which the pertinent stages of both taxa were sufficiently common (minimum 34 individuals counted, usually many more). In no comparison did the ratios of the abundances of the two taxa differ (contingency tests, $\chi_1^2 = 0.200–2.441$). We conclude that the overall avoidance of and retention by the coarse- and fine-mesh nets did not differ for these two taxa of different size, and accordingly that smaller organisms (at least those sufficiently wider than 64 μm) were captured with similar efficiencies by the fine-mesh nets.

The metered coarse-mesh samples were thus used to estimate field densities of copepodid stage IV through adult *C. finmarchicus*. Where these stages were too sparse in either the coarse-mesh or fine-mesh sample from a station (30+ individuals were deemed adequate, but often 100+ were counted), CV and adult stages of *Metridia lucens* (a few *M. longa* were included), and adults of *Centropages typicus* were added.

The estimated densities (no./m^3) of late copepodids of *C. finmarchicus* (and CV and adult *M. lucens* and adult *C. typicus* when the larger species was too scarce) based on the 333-μm nets could then be used to standardize the abundances of various smaller items in the fine-mesh samples. We aimed to use sufficiently large subsamples from the fine-mesh nets to give large counts (>30, often hundreds) of copepodids plus adults of the dominant smaller species. However, the catches were sometimes too scant for such counts from samples at stations along the shelf break. Only those copepod taxa (listed in the Results) that were found in all samples or otherwise pertinent to distribution or feeding of larval cod are considered here.

The 12 Clarke-Bumpus samples from the larval tracking cruise in late November and early December 1992 were treated similarly to the fine-mesh samples as described above. Sufficiently large plankton-splitter subsamples ensure that >100 copepodids and adults of the dominant copepodids (*C. typicus* in all samples) were obtained. All other calanoid copepodids and adults were counted in the same samples. Copepod eggs and nauplii, copepodids of *O. similis* and *M. norvegica*, and other common organisms (predominantly the pteropod *Limacina* sp.) were identified and counted (and some taxa measured) in 1/8 subsamples of these plankton-splitter subsamples. Because these tracking-cruise samples are not useful in delineating distributions or developmental patterns around Western Bank, only those species and stages that proved important in the associated larval cod diets are used here.

Laboratory analysis of stomach contents of cod larve

Cod larvae stored in liquid nitrogen were subsequently divided among several laboratories for treatment and analysis. For the present study, stomachs from frozen larval cod were removed and initially preserved in 70% ethanol, and later in 10% formalin, that provided superior preservation. Upon dissection, fullness of stomachs was visually estimated in five categories (i.e., nominally: empty, 25% full, 50% full, 75% full, or full). Identification and measurement of identifiable food items was done using fine dissecting needles and blades under dissecting and higher-power microscopes. Items that could not be identified to species or genus were assigned, when possible, pro rata within the taxa to which they belonged. Usually this involved animals identified as one of *Pseudocalanus* spp. or *P. parvus* or, sometimes, to either of these or *Clausocalanus* spp. Prosome lengths of copepodids were sometimes estimated by extension for missing parts.

The equivalent spherical diameters of copepodid prey were estimated from an equation fitted to the lengths and volumes of several species and stages listed by Herman and Mitchell (1981, their Table 1):

$$\ln \text{volume} = -2.640 + 2.665(\ln \text{prosome length})$$

Equivalent spherical diameters of predominant copepod nauplii were estimated from volumes calculated for various stages from measurements of prosome and urosome lengths and diameters, or of total lengths and diameters. A few nauplii of other species were assigned to the species with a similar naupliar shape. For nauplii of *Pseudocalanus* spp. (with *P. parvus*, *C. finmarchicus*, and *M. lucens* assigned),

$$\ln \text{volume} = -2.092 + 2.447(\ln \text{prosome length})$$

For *C. typicus nauplii*,

$$\ln \text{volume} = -1.989 + 3.063(\ln \text{total length})$$

Diameters of the few quasispherical pteropod, *Limacina* sp., consumed by the tracking cruise larvae were directly estimated from the stomach samples.

Statistical analyses

Relationships of numbers of prey in individual stomachs or stomach fullness with prey densities in associated plankton samples were analyzed by stepwise multiple regression to

Table 1. Spearman rank correlations of densities (no./m^3) of cod larvae with major copepod species (copepodids plus adults) expressed as densities and as percentages of total numbers of calanoids in each sample (*O. similis*, *M. norvegica*, and eggs and nauplii excluded; see text) on and around Western Bank, Scotian Shelf (too few larvae were caught during the November cruise for meaningful testing).

Correlation of larvae with Copepod species	December		January	
	Density	%	Density	%
Calanus finmarchicus	0.420	0.128	0.364	−0.140
Metridia lucens	0.336	0.291	0.571	0.211
Centropages typicus	0.622*	0.600	0.590	0.465
Temora longicornus	0.545	0.530	0.420	0.326
Pseudocalanus sp.	0.715*	0.669*	0.521	0.449
Clausocalanus sp.	0.442	0.411	0.345	0.105
Paracalanus parvus	0.550	0.594	0.759**	0.798**
Oithona similis	0.292	−0.463	0.410	0.026
Microsetella norvegica	0.254	−0.165	0.319	0.056
Copepod nauplii	0.410	0.224	0.261	−0.134
Copepod eggs[a]	0.218	−0.162	0.239	−0.390

Note: The correlations involved 17 stations in December and 20 in January. Significance levels (*, <0.05; **, <0.01) are based on Bonferroni correction for number of simultaneous correlations of each type within months.

[a] Excluding those carried by females.

remove the effects of other variables known or suspected to influence feeding rates of larval fishes. The significance level used to enter or remove variables was set at α = 0.15. This ensured that variables of interest were considered, even if they were not significant at traditional levels. Rationales for choices and transformations of variables, consideration of outliers, and other routine statistical procedures are given where appropriate in the Results.

Results

The major copepod taxa

Nine copepod taxa dominated the samples in late fall and winter 1991–1992. These were, in order of body size, the calanoids *Calanus finmarchicus*, *Metridia lucens* (a few *M. longa* included), *Centropages typicus*, *Pseudocalanus* spp. (mostly *P. newmani* with some *P. minutus*), *Clausocalanus* sp. (predominantly *cf. C. furcatus* with very few *cf. C. arcuicornis*, but, following Tremblay and Roff 1983, left unspecified), *Paracalanus parvus*, the cyclopoid *Oithona similis*, and the harpactacoid *Microsetella norvegica*. The calanoid *Temora longicornis* did not occur in all samples, but proved of interest in relation to patterns of circulation in the area. Although widespread, copepodids of *Calanus glacialis* and *C. hyperboreus* are not considered here, as they were relatively scarce and too large to be food for larvae. Other species found rarely or sporadically are not dealt with here. These included offshore, warmer-water forms found particularly at stations along the shelf break of Western Bank.

Distribution of cod larvae and zooplankton

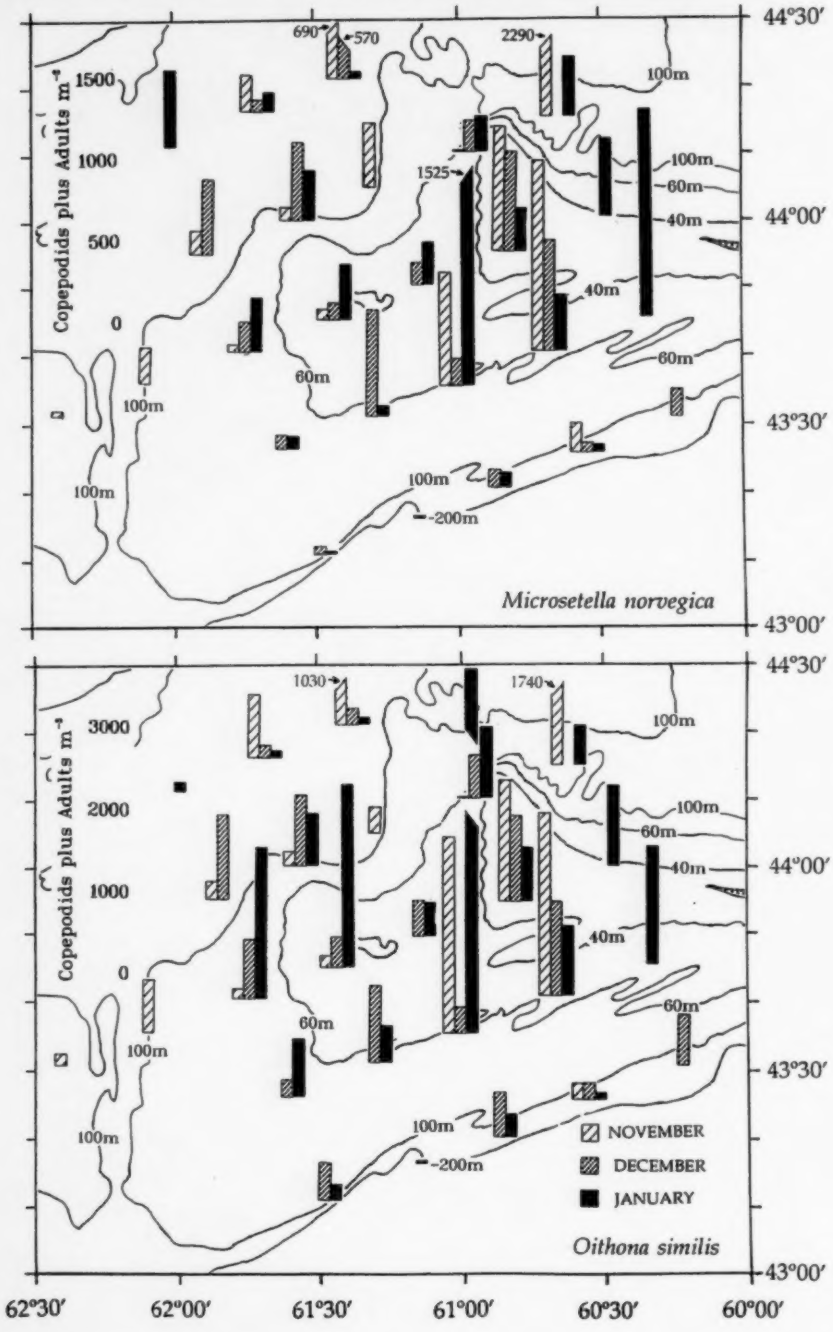
There was striking overall coincidence of cod larvae and copepod concentrations over Western Bank (Figs. 2, 3). Correlations of larval cod with copepod densities (Table 1) are all positive, significantly so for *Pseudocalanus* spp. and *P. parvus*. However, while these densities may reflect general hydrographic concentration mechanisms, they are also not statistically completely independent (both estimated from the same metered net samples). Expressing density of each species as a proportion of the total copepods in each haul removes this potential sample interdependence and also indicates their *relative* importance as associates of the cod larvae. In this index of relative importance (Table 1), *Pseudocalanus* spp. in December and *P. parvus* in January remained correlated with larval abundance.

As only the upper 75 m were sampled at deeper, off-bank stations, it is possible that larger copepod populations occurred at depth and remained undetected. However, the much lower densities of copepods off Western Bank (Figs. 2, 3) cannot be attributed to biased sampling in relation to possible vertical migrations. There was no day–night bias (start and end of night defined by local sunrise and sunset) in sampling times at deep (i.e., bottom >75 m versus shallow stations: 12/30 and 13/24 samples were collected at night at shallow and deep stations respectively (χ^2_1 = 1.141, ns). Furthermore, as usual (e.g., Skreslet 1989), most cod larvae occurred within the upper layers of the sampled depths (M.G. Meekan, personal communication), so their associations and dissociations with copepods (Table 1) are meaningful within the sampled water columns.

Distributions in relation circulation patterns

The distributions of both larvae and copepods are related to a recently discovered mean pattern of circulation, in which warmer, more saline water moves from the east along the southern edge of Sable Island Bank, then swings north along the western end of the Western Bank, forming a semi-gyre with colder, less saline water occupying an area of weaker currents over central Western Bank (Bowen

Fig. 3. Abundances of the two most common noncalanoids (copepodids plus adults) at stations on and around Western Bank during fall and early winter 1991–1992.



et al. 1995; K.R. Thompson, personal communication). Insights into possible biological consequences of this circulation around Western Bank are gained from two species with very different distributions. On the Scotian Shelf, *Temora longicornis* is an inshore species (Tremblay and Roff 1983), perhaps associated with outflow from the Gulf of St. Lawrence (Sameoto and Herman 1992). Its higher relative abundance over Western Bank and its strikingly

Table 2. Incidence and frequencies of identified prey items (copepodids plus adults except as noted) in 65 larval cod stomachs in December 1991 and 107 in January 1992 compared with abundances in the 10 plankton samples in each month at the same stations and on the same dates (the 23 stomachs from November contained too few identifiable items for meaningful comparisons).

	Stomach contents				In plankton (no./m^3 ± SE)	
	December		January			
Prey item	Incidence	Number	Incidence	Number	December	January
Pseudocalanus spp.	17	54	84	558	161±122	67±30
Paracalanus parvus	8	11	44	101	134±45	85±48
Centropages typicus	1	4	22	35	135±45	78±34
Oithona similis	1	1	16	30	665±108	1075±416
Calanus finmarchicus	0	0	12	21	24±5	29±3
Metridia lucens	1	2	7	8	82±36	36±11
Copepod eggs	0	0	1	9	129±36	180±73
Clausocalanus sp.	2	2	5	6	49±20	30±8
Copepod nauplii	2	2	2	5	190±40	202±87
Temora longicornis	0	0	3	4	7±3	19±12

Note: Prey items identified as one of *Pseudocalanus* spp., *P. parvus*, or *Clausocalanus* sp. (19 in December, 120 in January) were distributed pro rata among counts of identified individuals of these taxa (see Materials and methods). The harpactacoid (*Microsetella norvegica* was abundant (401 ± 65) in the plankton but not found in stomachs. Five *Limacina* sp. were found in stomachs but not fully assessed in the plankton samples.

low representation to the north, west and south (Fig. 4) apparently reflect advection from the northeast onto the bank. By contrast, *Clausocalanus* sp. is an offshore, warmer-water form, that spreads into shelf waters late in the year (Tremblay and Roff 1983). Although widespread in the present survey, it was generally proportionately most abundant at stations along the southern and western margins of Western Bank (Fig. 4), suggesting advection along the shelf break from waters to the northeast.

Further insights on the influence of this pattern of circulation on copepod distributions comes from the developmental stages of *Pseudocalanus* spp., an important correlate of cod larval abundance (Table 1). Their relative abundance in the central region of Western Bank could result either from increased productivity or from physical accumulation, or both. There were no strong differences within months in the stage–frequency distributions of copepodids and adults at stations on the bank compared with those "outside" (along its southern and western edges) or in deeper waters "inside" (to the north) of the bank (Fig. 5). There were also no consistently greater proportions of nauplii or adult males (that are very short lived), or more eggs per female, at on-bank stations, especially compared with those at deeper stations inside the bank (Fig. 5). These data together suggest that the abundance of *Pseudocalanus* spp. on the bank did not result from enhanced, in situ productivity (i.e., increased reproduction, development rate, or survival of this copepod), but rather from physical concentration within the semi-gyral centre of low residual currents.

Seasonal patterns of copepod abundance

The variances in copepod abundances, especially when all stations are considered, tended to obscure monthly patterns in abundance (Figs. 2, 3). Clearer trends emerge from stations with depths <75 m (i.e., bank stations sampled from near the bottom to the surface) during the period before and after the major spawning of cod (Fig. 6). Mean abundances of *O. similis* and *M. norvegica* changed little during the period (range 2.5-fold and 1.9-fold, respectively). *Paracalanus parvus* and *C. typicus*, both known to peak in late summer and early autumn (Tremblay and Roff 1983; McLaren et al. 1989), generally declined. *Calanus finmarchicus* increased 2.7-fold between October and January, partly through limited recruitment of early stages, but largely as later stages, presumably advected over the bank at the beginning of their return to surface waters for late winter spawning (McLaren et al. 1989; Sameoto and Herman 1990). The strong peak and decline of *Clausocalanus* sp. matches its known warm-water, late autumn abundance in previous studies (Tremblay and Roff 1983; McLaren et al. 1989). Larger and sustained increases occurred in *Pseudocalanus* spp. and *M. lucens* (both about 15× between October and December or January). Both copepod taxa are known to be more abundant in winter than in summer, beginning with larger cohorts in late autumn (Tremblay and Roff 1983; McLaren et al. 1989).

In conclusion, it appears that copepodids and adults of *Pseudocalanus* spp., already shown to be correlated with abundance of cod larvae on Western Bank (Table 1), also exhibited a timely and sustained increase in abundance coincident with cod spawning. Other species either showed no such large, sustained increases or, in the case of *M. lucens*, did so but were poorly correlated with larval abundance (Table 1). Copepod nauplii, that were uncorrelated with larval abundances in December and January (Table 1), were more abundant during November than during the subsequent two cruises (Kruskall–Wallace test statistic 9.22, $P = 0.010$; no difference between December

Fig. 4. Percentage abundances (of total calanoid copepodids and adults) of two species of copepods with different faunal affinities in samples from Western Bank during late fall and early winter 1991–1992.

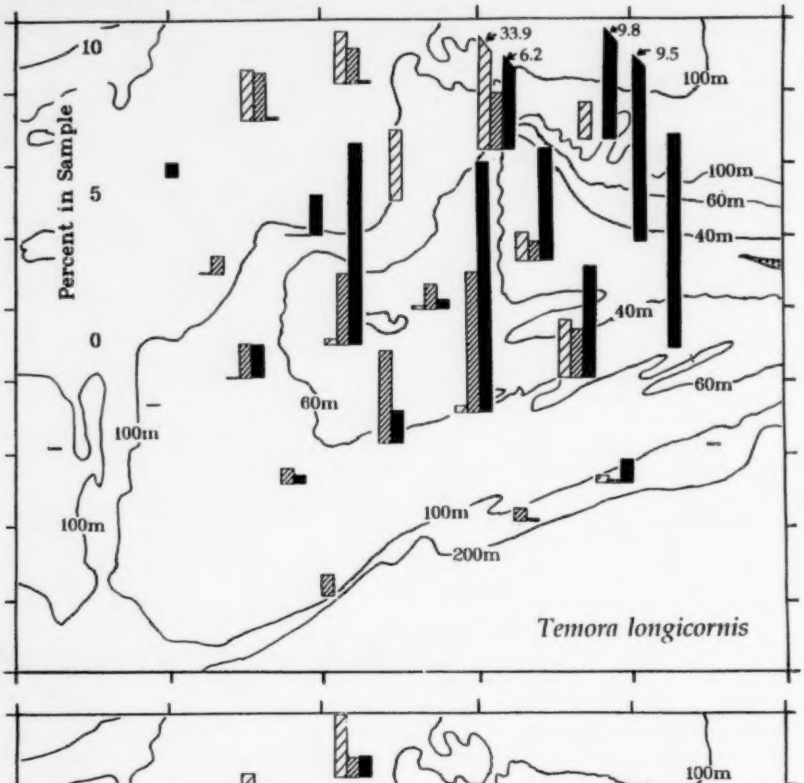
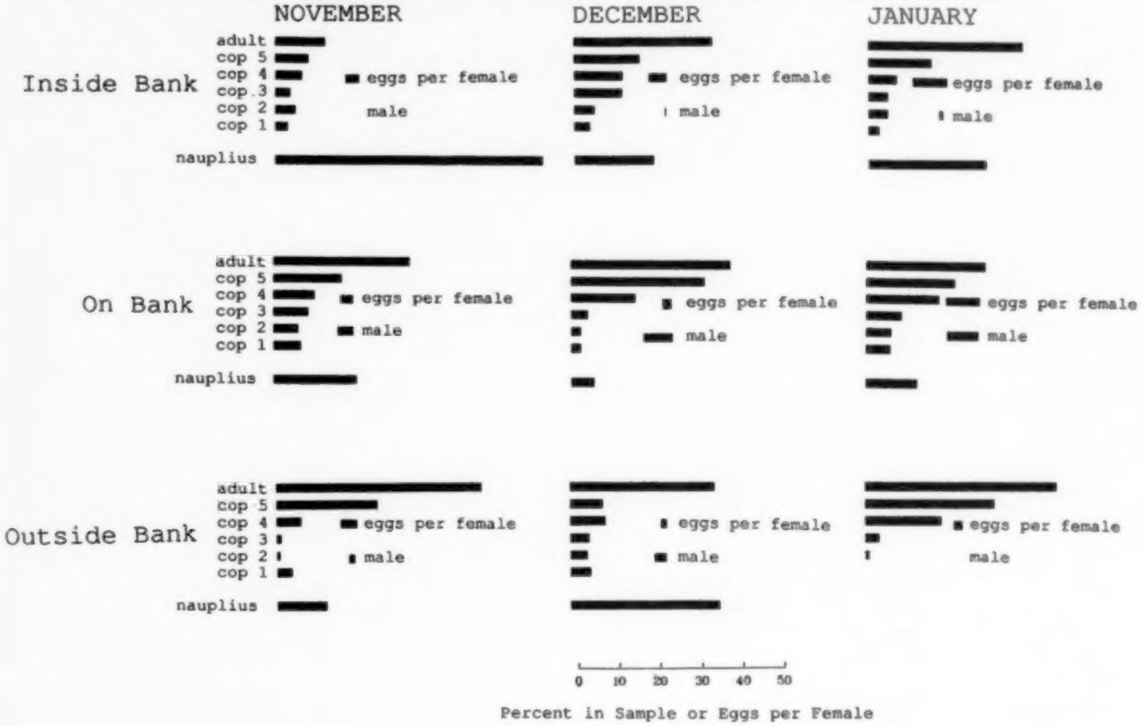


Fig. 5. Relative abundances of developmental stages of *Pseudocalanus* spp. (almost all *P. newmani*) at different locations on and around Western Bank (see text for positions of three categories) during late fall and early winter 1991–1992. The estimated numbers of nauplii in each sample were divided by 5 (because their 6 stages might occupy about 5 times the duration of a copepod stage) to make them more readily comparable with the proportions of each copepod stage.



and January, Mann–Whitney *U*-statistic = 128, $P = 0.25$). They were, however, uncorrelated with abundance of cod larvae during that month, although small sample sizes of larvae make this conclusion tentative.

Diets of larval cod

The 1991–1992 season

Copepodids and adults of *Pseudocalanus* spp. were the most abundant identified items in larval cod stomachs during the 1991–1992 season (Table 2). There is little value in calculating selectivity indices. It is obvious, for example, that the three most abundant items in the plankton (*M. norvegica*, *O. similis* and nauplii) were either never or seldom prey (Table 2). Also, *P. parvus*, that outnumbered *Pseudocalanus* spp. in most samples (Fig. 2), was less frequent as prey at all sample stations. A large number of the larvae from December (34 of 65) and January (64 of 107) came from a single station (nearest the 40-m contour "peak"; see lower panels of Fig. 2) sampled at several times over the 24-h day. *Paracalanus parvus* predominated over *Pseudocalanus* spp. at this station (ratios 2.6:1 in December, 1.7:1 in January) but was less frequent in stomachs (ratios 1:4.7 in December, 1:2.9 in January). *Pseudocalanus* spp. copepodids were clearly eaten out of proportion to their relative abundance.

It is more revealing to seek evidence of functional prey–predator relationships, while controlling for other variables believed a priori to influence feeding rates. The variables for stepwise multiple regression were selected and used as follows. (*i*) Cruise number was entered as a categorical variable, because larval history, age (partly independent of size), and feeding behaviour might have differed among months. (*ii*) As stomach capacity must scale as some power of length, larva lengths were log transformed. (*iii*) Feeding rates are presumably saturated at high prey densities. This effect was approximately linearized by using the logarithms of estimated prey densities, which indeed always gave smaller residual MS than did untransformed densities. (*iv*) Because cod larvae are known to have a diel feeding rhythm (e.g., Kane 1984), time of day was expressed as the deviation from the estimated peak time of feeding activity, which was iterated in repeated regressions to the nearest hour giving minimum residual MS with all variables. Incorporating squares of adjusted times, to account for possible nonlinearities in the daily feeding cycle, never added a significant effect. No interaction terms were significant in the various regressions explored.

Regressions were first estimated for stomach contents of all 195 larvae available from the three months from 36 samples at 13 stations, each with one to 64 larvae. (The latter came from the station where samples of larvae were

Table 3. Results of stepwise multiple regression of numbers of the two predominant prey copepods (copepodids plus adults) in 141 cod larvae <10 mm standard length during the cruises of November 1991 through January 1992, using size of cod larva, time (as deviation from best-fitted times of maximum fullness at 18:00 for *Pseudocalanus* spp.; time not significant for *P. parvus*), and abundance of the two taxa from the same plankton net samples.

Species	Effect	Value	df	MS	P
Pseudocalanus spp.	Cruise (category)[a]	—	2	41.816	0.011
	Adjusted time	−0.218	1	80.813	0.001
	ln larva length	0.715	1	19.716	0.116
	ln no./m^3	0.310	1	30.293	0.044
	Error	—	19	4.736	—
Paracalanus parvus	Cruise category[b]	—	2	1.519	0.046
	ln no./m^3	0.033	1	15.181	0.002
	Error	—	21	0.249	—

Note: Significance values are estimated using degrees of freedom conservatively based on the 25 plankton samples from the same stations and dates. Regression constants, other variables, and interactions had no effects at $P < 0.15$.

[a] Adjusted means: November, −0.020; December, 1.056; December, 2.342.

[b] Adjusted means: November, 0.003; December, 0.126; January, 0.379.

taken six times over a 24-h period in January; see above.) The unaggregated data produce properly weighted estimates of regression coefficients and mean squares, but significance levels are conservatively based on the 25 samples of plankton taken on the same dates at the same stations. Error variance from our inability to identify many food items should also give conservative correlations with prey densities in the field.

For this entire collection, only adjusted time of day and log larval length had significant influences on numbers of *Pseudocalanus* spp. in stomachs ($F_{1,22} = 12.21$, $P = 0.025$, and 57.07, $P < 0.001$, respectively). For *P. parvus*, only larval size was significant ($F_{1,23} = 30.95$, $P < 0.001$). As expected, the effects were negative for deviation from peak feeding time at 19:00 and positive for log length. However, the total sample included large (up to 19.7 mm) larvae that could have masked effects of food density on predation success of smaller larvae.

It is widely believed that small larvae are more sensitive to the quantity of food, because they lack either the strength or skills to find concentrations. For example, Lough and Potter (1993) found clear evidence of vertical migration only among larvae >9 mm, and 10 mm was the upper size of cod larvae analyzed for stomach contents by Kane (1984). In fact, such larger larvae are often designated as postlarvae (e.g., Suthers et al. 1989). Therefore, the regressions were estimated using larvae chosen arbitrarily (i.e., without optimization) as <10 mm in length. This cutoff provided 23 larvae from November (mostly small and with empty guts), 64 from December, and 54 from January. Sample month and prey densities influenced (at $P < 0.05$) numbers of the two predominant prey species in stomachs, and time of day and, marginally (at $P < 0.15$), larva size, affected feeding on *Pseudocalanus* spp. (Table 3). No significant effects of prey density were evident in regressions using the other species in Table 1. It is reassuring that the fitted time of peak feeding (19:00) by larvae on *Pseudocalanus* spp. was close to the peak time of feeding found; by Kane (1984, Fig. 7; ca. 18:00) for cod larvae on Georges Bank. Inevitably with such material, there were statistical outliers (six data points) in both regressions, but none exerted significant leverage on the regression results.

The late-1992 season

The tracking cruise of late 1992 was designed partly to obtain more precise associations between samples of stomach contents and potential prey. As noted in the Materials and methods, zooplankton was sampled at four fixed depths, while larvae came from strata within 5 m above and below these depths at the same stations. All samples were taken in the region of high copepod density on Western Bank (cf. Fig. 1, 2) closer to peak feeding times (see above).

The tracking-cruise larvae from which stomachs were sampled were much smaller (mean length ± SD = 4.82 ± 1.09 mm) than those sampled during the 1991–1992 season (11.73 ± 4.14 mm), and their diet consisted mostly of nauplii (Table 4). The larvae were clearly selective: for example, the small copepodids of *O. similis* and nauplii of *C. typicus* were more abundant than nauplii of *Pseudocalanus* spp. in all the plankton samples, while the last predominated in stomachs.

In the stepwise regressions, larval size and depth are a priori of potential influence on the prey–predator relationship. As all four sample series were taken in the afternoon, time of day was not expected to be a detectable effect, and in fact was not significant in any regressions.

Among the four most abundant prey (Table 4), consumption of nauplii of *Pseudocalanus* spp. *C. typicus* was strongly related to their densities in the water column (Table 5). However, this was not true (at $P < 0.05$) for *Pseudocalanus* spp. copepodids or small individuals (those eaten were <250 μm in diameter) of the pteropod *Limacina* sp. Depth of sample strongly influenced feeding on *Pseudocalanus* copepodids

Table 4. Aggregate frequencies of identified prey items in 177 larval cod stomachs, from the larval tracking cruise on Western Bank, 28 November to 2 December 1992, compared with abundances in the plankton at the stations and depths at which cod larvae were captured.

	In stomachs		In plankton
	Incidence	No. of items	(no./m^3 ± SE)
Pseudocalanus spp. nauplii	162	965	364±113
Centropages typicus nauplii	150	694	1010±205
Pseudocalanus spp. copepodids	93	323	98±12
Limacina sp. (<250 μm diameter)	31	120	1360±445
Oithona similis copepodids	23	27	1467±188
Other nauplii[a]	17	24	421±75
Other small copepodids[b]	6	1	114±10
Centropages typicus copepodids	3	3	1239±85
Paracalanus parvus copepodids	2	2	411±62

[a] Mostly *Metridia* spp. in stomachs, *O. similis* in plankton.

[b] Mostly *Clausocalanus* sp., *T. longicornis* in plankton.

Table 5. Results of stepwise multiple regression of numbers of three predominant prey items in 177 cod larvae from four depths at four stations during the larval tracking cruise in late November and early December 1992, using length of larva, depth of sample, and abundance of the prey items at about the same times and depths (see text).

Prey item	Effect	Value	df	MS	*P*
Pseudocalanus nauplii	Depth (m)	0.076	1	110.55	0.089
	ln no./m^3	0.799	1	1160.35	<0.001
	Error	—	9	19.53	—
C. typicus nauplii	Depth	0.096	1	148.44	0.046
	ln no./m^3	0.033	1	312.57	0.002
	Error	—	9	15.15	—
Pseudocalanus copepodids	Constant	−11.456	1	407.60	<0.001
	Depth	0.076	1	554.36	<0.001
	ln larva length	0.799	1	22.92	0.121
	Error	—	9	4.82	—
Limacina sp. (<250 μm)	Constant	6.842	1	24.64	0.004
	Depth	−0.035	1	5.02	0.129
	ln larva length	−2.119	1	10.30	0.034
	ln no./m^3	−0.353	1	6.17	0.096

Note: Degrees of freedom are conservatively based on the 12 plankton samples available for comparison. Other regression constants, variables, and interactions had no effect at $P < 0.15$.

are perhaps weakly so for other prey. Larval size had a weakly positive effect on the use of *Pseudocalanus* copepodids and a significantly negative effect on the use of *Limacina* sp. There were only two statistical outliers in the regression for *Pseudocalanus* nauplii, and five in that for *C. typicus* nauplii, none of which had significant leverage on the regression coefficients. However, the regressions for *Pseudocalanus* copepodids and *Limacina* sp., which involved smaller samples (Table 4), were less reliable, with four outliers exerting undue influence in the former regression, and two in the latter.

The following general conclusions seem possible from analyses of diets in the 1992 season. (*i*) Even in these waters with relatively high prey concentrations, density of prey was important to small larvae feeding on nauplii, but possibly not when they switched (when somewhat larger?) to copepodid prey. (*ii*) *Limacina* sp. tended not to be eaten by larger larvae and was possibly avoided when at large densities. (*iii*) Feeding was generally more active (except on *Limacina* sp.) in deeper strata within the 5–35 m range.

Sizes of prey

It is well known that size of prey eaten increases with size of larval fishes (e.g., Kane 1984). However, there was no evidence of this in equivalent spherical diameter of prey (ESD; see Materials and methods) of larval cod from the 1991–1992 season (Fig. 7; ESD = 0.343 − 0.001·length, $F_{1,844} = 1.590$, ns). Most prey of all sizes during that season

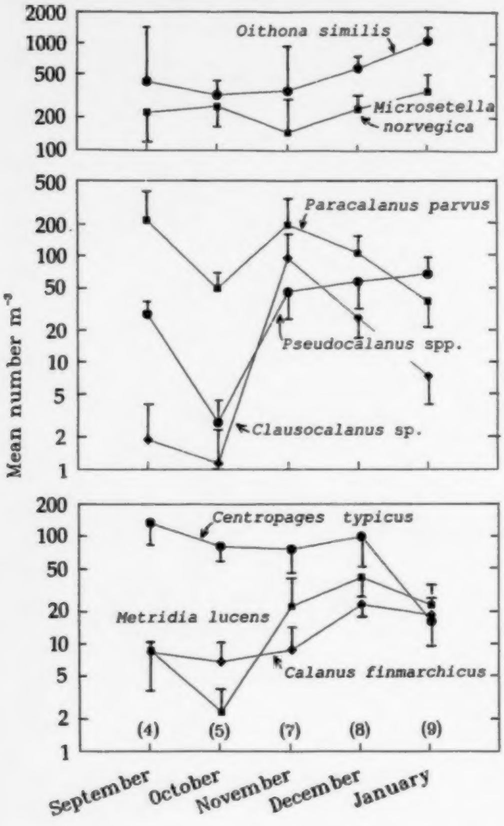


Fig. 6. Numbers of copepodids plus adults of predominant copepods at stations with depths <75 m on Western Bank during fall and early winter 1991–1992. Numbers of stations sampled are listed above each month. Error bars are 1 SE, one-sided for clarity.

were between 0.2 and 0.5 mm ESD, which represents a range equivalent to prosome lengths of copepodid stages of *Pseudocalanus* spp. This is not surprising as this taxon predominated in the diets (Table 2). The few large items (>0.6 mm ESD) were equivalent to small copepodids of *C. finmarchicus*.

Most larval cod collected during the tracking cruise in late 1992 ate nauplii, although copepodids of *Pseudocalanus* spp. may have been favoured by larger larvae (Table 5). Thus, a positive relationship between size of prey and size of larvae is expected (ESD = $-0.002 + 0.033 \cdot$length, $F_{1,496} = 195.1$, $P < 0.001$). The combined data from the 1991–1992 and 1992 season (Fig. 7) indicate that larvae longer than 5–6 mm could use larger prey.

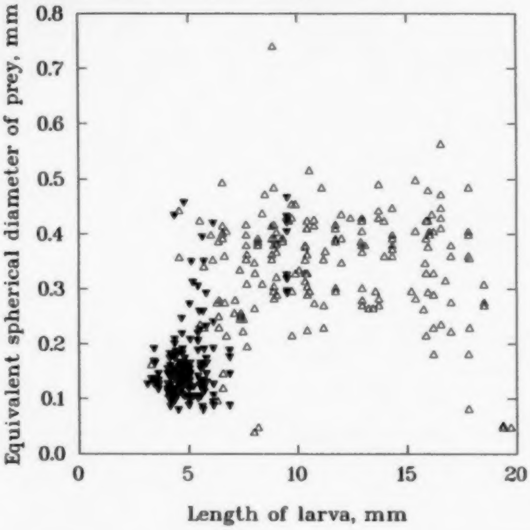


Fig. 7. Equivalent spherical diameters (see Materials and methods) of measurable food items in stomachs of larval cod from Western Bank (open symbols for the 1991–1992 season, solid ones for late 1992). A random subsample of 200 measured items from each season (844 measured from 1991–1992, 498 from 1992) is displayed for clarity, but regressions (see text) were done on the complete data sets. The larval lengths are for fresh specimens, somewhat longer than preserved specimens.

Fullness of stomachs

Fullness of stomachs was qualitatively assessed (in five categories) even when they contained much unidentifiable material. The 195 stomachs accompanied by copepod prey densities from 25 samples in 1991–1992 averaged slightly less than half full (mean ± SE = 41 ± 1.5% full). The numbers of food items (almost all copepodid and adult copepods) in 31 stomachs rated as full depended on larval size. Regression (ln[no. items] = 0.723 + 0.823 ln[larva length], $P = 0.002$) suggests that an average 5-mm larva could consume at least 9 items of similar size, and a 20-mm larvae at least 29 items of similar size.

Stepwise regression with all 194 stomachs samples and the variables used for analysing diets (see previous sections) revealed important effects of month ($P = 0.009$), larval size ($P = 0.013$), and time of day ($P < 0.001$), but eliminated interaction terms and all prey items except *P. parvus* ($P = 0.047$). However, field densities of *P. parvus* and *Pseudocalanus* spp. were very strongly correlated and, in separate regressions, produced almost identical effects ($F_{1,19} = 6.560$ and = 6.117 respectively, both $P < 0.05$), so that the elimination of the latter species as a variable is highly questionable.

Again it can be argued that smaller larvae (many with empty stomachs) were more sensitive to food levels than larger ones. Stepwise regressions using 54 larvae >10 mm long eliminated all predictors of stomach fullness except time of day. However, regressions for 141 larvae <10 mm long eliminated *P. parvus* as a predictor (*P* only ca. 0.90), but sanctioned the regression of Table 6, with a highly significant effect of *Pseudocalanus* spp. densities. Although the explanatory power of this regression was quite high ($R^2 = 0.538$), and there was only one statistical outlier, some anomalies inevitably remained. For example, above-average fullness values were found among eight larvae taken at two off-bank

Table 6. Multiple regression of fullness of stomachs (five categories, nominally 0–100%) of 141 cod larvae <10 mm in standard length during the cruises of November 1991 through January 1992, using larval size, time (as deviation from best-fitted time of maximum fullness at 19:00), and abundance of *Pseudocalanus* spp. at the same stations (other species and various interactions not significant; see text).

Variable	Value	df	MS	*P*
Cruise category[a]	—	2	4716	0.020
ln larva length	19.117	1	5143	0.014
Time of day	−3.798	1	21787	<0.001
ln *Pseudocalanus*/m^3	3.025	1	7548	0.003
Error	—	19	546	—

Note: Degrees of freedom are conservatively based on the 25 stations sampled (see text).

[a]Adjusted means: November, 11.355; December, 27.928; January, 44.471.

Table 7. Multiple regression of fullness of stomachs (five categories, nominally 0–100% full) of 177 cod larvae from the larval-tracking cruise in late 1992, using depth, larva size, and abundance of prey at about the same stations and depths.

Variable	Value	df	MS	*P*
Constant	66.588	1	9772	<0.001
Depth	0.557	1	3573	0.084
ln *Pseudocalanus* nauplii/m^3	8.763	1	6708	0.021
ln *Limacina* sp./m^3	−7.334	1	7204	0.017
Error	—	7	538	—

Note: Other variables and interactions not significant at $P < 0.15$. Degrees of freedom are conservatively based on the 12 plankton samples available for comparison.

stations with the lowest levels of *Pseudocalanus* among the samples used. Although interaction terms were not significant, the effects of cruise month presumably derived from different qualities among larvae or of depth or patch relationships among larvae and prey over the 3 months. Omitting cruise as a variable retains the effects of time of day ($F_{1,20} = 52.773$, $P < 0.001$), *Pseudocalanus* density ($F_{1,20} = 7.676$, $P = 0.020$), and especially length ($F_{1,20} = 64.878$, $P < 0.001$), the last presumably reflecting the importance of increasing average size of the (<10 mm) larvae in successive cruise months.

Larvae sampled during the 1992 tracking cruise had fuller stomachs (mean = 73 ± 1.8% full) than those obtained during the 1991–1992 monthly series (see above). This is expected, because the former were taken in the afternoon at near-peak feeding times in a region of high prey densities. The relationship for 64 larvae rated as "full" between number of items and body size is: (ln[no. items] = 1.530 + 0.911 ln[larva length], $P = 0.012$). This implies that a 5-mm larva on average could consume up to 20 prey. This number, largely based on nauplius-sized items, is of course larger than that estimated from the larger larvae, consuming larger prey, in 1991–1992 (see above).

Stepwise analysis of variables influencing stomach fullness of the tracking-cruise larvae produced clear results (Table 7). Within the narrow size range of the small larvae sampled, their length had no influence on fullness. As with the feeding rates on most major prey (Table 5), stomach fullness was weakly related to depth. Of the four major prey items (Table 4), only densities of *Pseudocalanus* nauplii had a positive effect on stomach fullness. The significantly negative effect of *Limacina* sp. suggests that this item was avoided at high densities. No statistical outliers were revealed in this regression analysis.

The results on fullness of stomachs from both seasons thus strongly support the association between cod larvae and their predominant copepod prey, especially *Pseudocalanus* spp., as demonstrated in previous sections. Although, inevitably, more stomachs that contained high concentrations of copepods, including *Pseudocalanus* spp., were available from the 1991–1992 stations, this in itself would not have the produced positive effects of densities of this copepod on stomach contents and fullness. Certainly the tracking-cruise results are free of such effects: *Pseudocalanus* nauplii were never predominant in plankton samples but were the only taxon with a positive effect on stomach fullness (Table 7).

Discussion

The earliest samples of cod larvae collected in 1991 did not reveal a dependence on a large burst of nauplii. Nauplii of all species at the four stations from which larvae were obtained in November 1991 were not notably more abundant (m^{-3}: mean ± SE = 1489 ± 534) than copepodids and adults (calanoid species combined, 906 ± 317; *O. similis*, 1528 ± 546; *M. norvegica*, 655 ± 240) at the same stations. However, no nauplii were found in stomachs of the 23 larvae available from November 1991. Of the two favoured prey in the plankton in late 1992 (Table 5), nauplii of *Pseudocalanus* spp. on average outnumbered their copepodids by >3:1, and nauplii and copepodids of *C. typicus* were about equally common (Table 4). Although early nauplii or eggs of *C. finmarchicus* have been implicated as primary food for cod larvae (e.g., Ellertsen et al. 1989; Skreslet 1989; review by Runge 1988) and were possibly involved in spring spawning of cod on the Scotian Shelf in earlier years (Brander and Hurley 1992), this copepod was neither abundant nor in suitably early stages during the 1991 fall spawning on Western Bank. It may be significant that most of the small larvae during November 1991 had empty stomachs. Evidently the larger larvae found in later months were able to satisfy their needs on copepodids.

The dependence of stomach fullness on *Pseudocalanus* spp. (mostly *P. newmani*) in both sample seasons (Tables 6, 7) was particularly striking, and not subject to possible biases from differential digestion rates, errors of identification of prey, etc. *Pseudocalanus* spp., uniquely among the smaller copepods, showed a large, sustained increase in abundance from November through January (Fig. 5). It is tempting to hypothesize that the late-autumn spawning of cod on Western Bank is adapted to a late-autumn generation of *P. newmani* in the region (cf. Fig. 10 in McLaren et al.

1989). Among the copepodids of comparable size, those of *Pseudocalanus* spp. store more evident lipid (in a conspicuous "oil sac") than do *P. parvus* and *Clausocalanus* sp. and may offer greater food value. They are also less robust and spinous than the approximately equally abundant (Table 2) *C. typicus*. These and other reasons, such as swimming behaviour, for the apparent choice of *Pseudocalanus* spp. could usefully be investigated.

The mean water-column concentrations of copepods, including *Pseudocalanus* spp. (Table 2), were lower than those found to produce near satiation in larval cod off Norway (Ellertsen et al. 1984, Fig. 4) and much lower than those often used in feeding experiments (Otterä 1993). The true prey densities for feeding larvae cannot be inferred from the vertically integrated plankton samples taken in 1991–1992. However, the 1992 tracking-cruise results did not reveal striking concentrations at any one depth. Possibly *Pseudocalanus* spp. were concentrated at much smaller spatial scales, but any such microscale patchiness did not mask the positive prey–predator relationships demonstrated in our analyses (Tables 3, 5, 6, 7). It is of interest that Winemiller and Rose (1993) have shown theoretically that production of large numbers of small larvae by fishes such as cod is an adaptation to conditions where food is randomly distributed or only moderately clumped.

Although the regression coefficients describing dependence of diets or stomach fullness on prey densities are probably in acceptable functional form (log densities implying a saturation function), they could not be used for predicting the adequacy of a prey field for support of feeding of larval cod. A detailed functional prey–predator relationship for larvae and their prey in the field might only be established by highly repeated samples of small water volumes, perhaps by pumps or bottle samplers. However, such a pursuit may be questionable. It is clear that diets and stomach fullnesses in the 1991–1992 samples reflected the very large differences between prey abundances on and off Western Bank. It may be sufficient to assume that nutrition is adequate on, and inadequate off, the bank.

On this larger scale, the importance of copepods to cod larvae is clearly related to the coincidence of both on Western Bank. This concentration appears to involve advection of copepods onto the bank from the northeast (Fig. 4). It is not certain that the high numbers of cod larvae resulted from spawning on the bank or from similar advection onto the bank. If the latter, there may have been an unsampled spawning area in the bathymetrically complex region north and northeast of Sable Island, although this was not clear in earlier years (see Fig. 2 in Frank et al. 1995).

The results have implications for planning future studies of feeding and predation by fish larvae and for interpreting earlier work. (*i*) The associations and relationships between larvae and their prey could not have been discovered without fine-mesh nets which, as noted by Frank (1988), have been too seldom used in previous fish larval surveys. (*ii*) Size alone may not determine prey choice, given that *Pseudocalanus* spp. were favored among a range of like-sized items. Abundance of size fractions of zooplankton (e.g., Bollens et al. 1992) may not reveal availability or suitability of food items, and particle-counting methodologies for rapid assay of prey fields should be accompanied by attempts to identify the organisms involved (as by Herman and Mitchell 1981). (*iii*) Larval cod rearings have assumed that small larvae prefer or require very small food particles. In experiments, the early use of very high concentrations of nauplii and eggs of smaller species and the withholding of larger items until later (e.g., Otterä 1993) may not match the needs of cod larvae. The present results (e.g., Fig. 7) suggest that larvae longer than 5–6 mm can use, and may prefer, larger items.

Finally, the results may also have strategic implications for work needed to improve our understanding of recruitment of cod and other bank-spawning groundfishes. Study of the mechanisms that maintain high (satiation level?) densities of prey for larvae may be more important than further specification of functional prey–predator relationships, either experimentally or in the field. The roles of offshore banks as spawning areas may not be fully evident without further understanding of associated frontal and gyral mechanisms in concentrating larval food. More study of the behavior of zooplankton in surrounding waters, especially their vertical positionings and migrations, may provide insights into the interactions with physical conditions that lead to their accumulation as potential food items on banks. This would require carefully positioned samplings on finer scales of time and space than those used in the present study. It may also be that recruitment failure is driven, not by seasonal or local mismatches between spawning and particular prey species on such banks, but rather by the breakdown of associated gyre systems throughout the season when larvae depend on high levels of prey. This could involve storm-driven events that occur long after the supposed "critical period" during the switch from yolk-sac dependence. A deeper understanding of such mechanisms could reveal appropriate physical or biological variables, perhaps amenable to remote sampling, to help predict year-to-year variations in groundfish recruitment.

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Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota

Todd M. Koel and John J. Peterka

Abstract: Laboratory-based bioassays were conducted to determine concentrations of sodium-sulfate type salinities that limit the hatching success of several fish species. Survival to hatching (SH) was significantly lower ($P < 0.05$) in sodium-sulfate type waters from Devils Lake, North Dakota, of ≥2400 mg/L total dissolved solids (TDS) than in fresh water of 200 mg/L. In waters of 200, 1150, 2400, 4250, and 6350 mg/L TDS, walleye (*Stizostedion vitreum*) SH was 41, 38, 7, 1, and 0%; northern pike (*Esox lucius*) SH was 92, 68, 33, 2, and 0%; yellow perch (*Perca flavescens*) SH was 88, 70, 73, 0, and 0%; white sucker (*Catostomus commersoni*) SH was 87, 95, 66, 0, and 0%; common carp (*Cyprinus carpio*) SH was 71, 69, 49, 63, and 25%.

Résumé : À partir du laboratoire, nous avons mené des bioessais en vue de déterminer, dans les salinités de type sulfate de sodium, quelles sont les concentrations qui limitent le succès d'éclosion de plusieurs espèces de poissons. La survie jusqu'à l'éclosion (SH) était significativement plus basse ($P < 0,05$) dans des eaux de type sulfate de sodium provenant du lac Devils, dans le Dakota du nord, qui contenaient ≥2400 mg/L de solides dissous totaux (TDS) que dans de l'eau douce qui en contenait 200 mg/L. Dans des eaux contenant respectivement 200, 1150, 2400, 4250 et 6350 mg/L de TDS, la SH du doré jaune (*Stizostedion vitreum*) était de 41, 38, 7, 1 et 0%; celle du grand brochet (*Esox lucius*) était de 92, 68, 33, 2 et 0%; celle de la perchaude (*Perca flavescens*) était de 88, 70, 73, 0 et 0%; celle du meunier noir (*Catostomus commersoni*) était de 87, 95, 66, 0 et 0%; enfin, celle de la carpe (*Cyprinus carpio*) était de 71, 69, 49, 63 et 25%.
[Traduit par la Rédaction]

Introduction

Saline lakes of the Upper Great Plains in the United States and in Canada have been managed to provide sport and commercial fisheries (Huntsman 1922; Rawson and Moore 1944; Atton 1986). While growth of fishes is often excellent (Rawson 1946; Marshall and Johnson 1971), natural reproduction is often restricted because salinity tolerance of embryos and sac fry is lower than that of adults (Atton 1986). Reviews of the effects of salinity on fish reproduction by Alderice (1988), Billard et al. (1981), and Holliday (1969) consider effects of sodium-chloride waters but do not address effects of sulfate waters that are characteristic of saline lakes of the Upper Great Plains.

Ionic composition of waters has a marked effect on hatching success of fish. Burnham and Peterka (1975) found that embryos and sac fry of fathead minnows (*Pimephales promelas*) survived only in sodium-sulfate waters of <8000 μS, but 68% survived in sodium-chloride waters of 18 000 μS. Salinities required for fertilization and (or) hatching of fish living in sodium-sulfate waters are difficult to determine because salinity varies seasonally (Atton 1986) and because most saline lakes are supplied by freshwater inflows that provide a wide range of salinities for spawning of fishes. Documenting natural reproduction of fishes in saline lakes is often further confounded by annual stocking of fish for sport or commercial fisheries (Hammer 1986).

Even though Hendrickson (1990) and Violett (1992) established that reproduction of most game fishes occurred in western areas of the Devils Lake chain in northeastern North Dakota, the absolute limits of water salinities could not be determined. Further, the absence of YOY may have been caused by lack of spawning habitat and (or) too few adults. The primary objective of our study was, by the use of laboratory bioassays, to determine the upper limits of sodium-sulfate saline waters that restrict the survival to hatching (SH) of walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), yellow perch (*Perca flavescens*), white sucker (*Catostomus commersoni*), and common carp (*Cyprinus carpio*). Laboratory experiments were not confounded by natural fluctuations in lake water levels or temperatures, the availability of suitable spawning habitat, or stocking of fishes.

Materials and methods

Water collection

Water used in bioassays was collected in early spring 1992 from East Devils Lake and Devils Lake (Six Mile Bay

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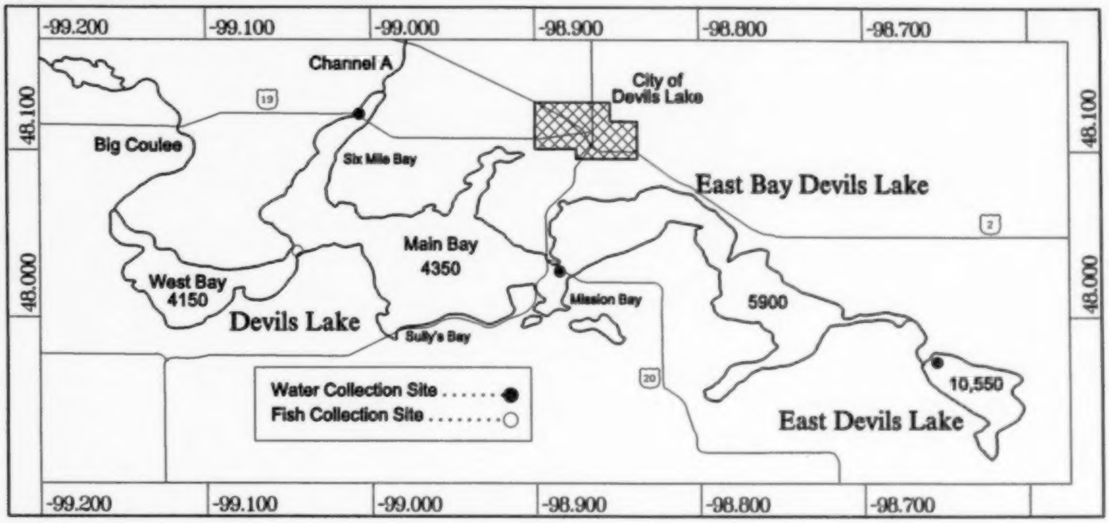
Fig. 1. The Devils Lake chain of lakes, North Dakota. Numbers indicate concentrations of total dissolved solids (mg/L) in summer 1992.



and Mission Bay) (Fig. 1). The water was filtered through a nylon screen with mesh size of 37 μm and stored in covered 18-L polyethylene buckets at 5–10°C. Dechlorinated tap water (fresh water) with a TDS of 200 mg/L from Fargo, North Dakota, was used as the control and to dilute Devils Lake water when needed to obtain desired treatment conductivities. Conductivity (μS/cm at 25°C) was measured using a Lab-Line Instruments, Inc. Lectro mho metre and converted to TDS (mg/L) by

$$\text{TDS} = -371.01 + 0.8394\ (\text{conductivity})$$

TDS and conductivity data for Devils Lake were obtained from the United States Geological Survey (1951, 1974, 1978, 1984, 1986). TDS ranged from 1750 to 6700 mg/L and correlation with measured conductivity was good ($r = 0.992$, $n = 5$). Chemical analysis of waters used in the bioassays was provided by the North Dakota State Department of Health and Consolidated Laboratories, Bismarck, North Dakota.

Spawning procedure

Eggs were obtained from walleye, northern pike, and yellow perch captured in frame nets set from mid-April to early May 1992 during the spawning run in Main Bay, Devils Lake (Fig. 1). White sucker eggs were obtained during the spawning run in May 1992 from Many Point Lake, Becker County, north-central Minnesota. Eggs from several females were pooled and fertilized in the field in fresh water (dechlorinated Fargo tap water used as a control) of 200 mg/L TDS and in saline waters from Devils Lake of 1150, 2400, 4250, and 6350 mg/L. Milt was collected and held in a syringe to assure immediate fertilization once eggs were exposed to treatment water. Eggs and milt were added to a round polyethylene bowl (17 cm diameter and 8 cm deep) containing about 250 mL of treatment water maintained at ambient lake water temperatures. After swirling the water and allowing a few minutes for fertilization to occur, several hundred eggs were placed in rectangular polyethylene trays (17 × 12 × 5 cm deep) containing water of the same salinity used for fertilization. The trays were placed in coolers maintained at 8–10°C with ice and transported to the laboratory. Common carp were netted during June 1992 from the Red River near Fargo, North Dakota. Spawning was induced with injection of aqueous extracts of fresh carp pituitaries. Devils Lake waters were used for fertilization of carp eggs at 20°C.

Fertilization success

Fertilized eggs were held overnight in their respective transportation trays prior to placement in egg incubation baskets. Live embryos were identified by normal cell division of the blastodisc within 1 d after fertilization for northern pike, walleye, and common carp and at 3 d after fertilization for yellow perch and white sucker. At least 100 eggs of each species were examined to determine fertilization success (FS) for each treatment salinity. FS was determined as the number of live embryos/number of eggs that were fertilized.

Hatching success

Live embryos were placed in small plastic petri dishes (egg baskets) with nylon screen (0.3-mm openings) along the side within 20–30 h after fertilization for incubation. Egg baskets containing 40–60 eggs each were placed in water in test chambers (glass aquaria) and positioned on glass plates near the surface of the chambers. Test chambers contained dechlorinated Fargo tap water of 200 mg/L TDS or Devils Lake waters of 1150, 2400, 4250, or 6350 mg/L. Water in the chambers was regularly replenished to maintain constant conductivity. An airlift system dripped oxygenated test water into the egg baskets from horizontal PVC tubes (one tube along each side of a test chamber) with holes positioned over the egg baskets.

Table 1. Chemical characteristics of waters from Fargo, North Dakota, and various sites in the Devils Lake basin used in hatching success bioassays.

Source	Na	Mg	K	Ca	Cl	SO_4	CO_3	TDS	Conductivity
Fargo[a]	25.6 (12.7)	7.1 (3.5)	10.6 (5.3)	32.0 (15.9)	22.3 (11.1)	49 (24.4)	54.6 (27.1)	191	365
Six Mile Bay[b]	384 (23.7)	94.7 (5.8)	43.7 (2.7)	25.5 (1.6)	164 (10.0)	806 (48.9)	124.8 (7.6)	1640	2490
Mission Bay[b]	1030 (23.2)	216 (4.9)	119 (2.7)	53.1 (1.2)	508 (11.4)	2220 (50.0)	289.8 (6.5)	4440	6200
East Devils Lake[a]	1680 (25.3)	326 (4.9)	168 (2.5)	48.3 (0.7)	737 (11.1)	3430 (51.6)	251.4 (3.8)	6630	8590

Note: Concentrations of nutrients and ions are expressed as milligrams per litre with percent of total dissolved solids (TDS) in parentheses. Conductivity is expressed as microsiemens per centimetre at 25°C.

[a] Analysis conducted during 1992.

[b] Analysis conducted during 1991.

Two insulated cooling tanks (Living Stream LS-700), each holding up to nine test chambers, were used to maintain a water bath of constant temperature. Water bath temperatures for walleye, northern pike, and yellow perch eggs were maintained at 11.5 ± 0.8°C. White sucker and common carp eggs were incubated at 15.0 ± 0.1 and 19.9 ± 0.7°C, respectively. Live and dead embryos were counted every 1–3 d until the date of hatching using a dissecting microscope and water bath to control temperature. Dead embryos, removed to prevent the spread of fungus, typically were identified as dark or opaque with abnormal cell development. Hatching success (HS) was taken as the number of embryos that hatched/number of live embryos, and survival to hatching (SH) was computed as the product (FS)(HS).

Statistical analysis

Statistical tests were done using SAS (Statistical Analysis System; SAS 1990) with α set at 0.05. Percentages were normalized by arcsine transformation (Sokal and Rohlf 1981) and analyzed using a single-factor analysis of variance (ANOVA). Duncan's Multiple Range Test was used for comparisons among means.

Results

Chemical characteristics of water

Sodium and sulfate were the dominant ions in Devils Lake waters. The relative percent composition of major ions was similar in all waters used; sodium was 23.7–25.3%, sulfate 48.9–51.6%, and chloride 10.0–11.4% of TDS (Table 1).

Calcium, carbonate, and sulfate were the dominant ions in dechlorinated Fargo tap water used as a control. Calcium was 15.9%, carbonate 27.1%, sulfate 24.4%, and chloride 11.1% of TDS (Table 1).

Hatching success of fishes

Walleye FS, HS, and SH were similar in the control water of 200 mg/L TDS and in saline water of 1150 mg/L, but markedly declined at salinities ≥2400 mg/L (Table 2). SH was only 7% at 2400 mg/L, <1% at 4250 mg/L, and no hatching occurred in water of 6350 mg/L TDS (Fig. 2). SH in waters with TDS ≥2400 mg/L was significantly lower ($P < 0.05$) than in the control. The low FS of 53% in the control was probably due to poor egg quality because of low water temperatures during the spawning run. Most embryo mortality occurred in all waters during the first 4 d of the 19 d incubation period (Fig. 3). All embryos incubated in water of 4250 mg/L had died or hatched by 17 d following fertilization.

Northern pike FS, HS, and SH were excellent in the control, but declined with increasing sulfate salinity (Table 2). SH of northern pike embryos was 92% in the control but was only 2% at 4250 mg/L TDS (Fig. 2). No embryos hatched in water with TDS of 6350 mg/L. SH in all sulfate-saline waters was significantly lower than in the control ($P < 0.05$); both FS and HS decreased in waters with TDS ≥1150 mg/L. Survival of embryos declined gradually over the 11-d incubation period with high mortality occurring between days 6 and 7 in waters with TDS of 2400 and 4250 mg/L (Fig. 3).

Yellow perch FS was 100% in the control and in saline waters with TDS of 1150 and 2400 mg/L, but low HS reduced SH in waters of 1150 mg/L and 2400 mg/L (Table 2). All eggs were dead within 3 d of fertilization in waters with TDS of 4250 and 6350 mg/L either because of lack of fertilization or development thereafter. SH in all sulfate-saline waters was significantly lower ($P < 0.05$) than in the control (Fig. 2). Embryo survival during the 14-d incubation period was good in those waters where successful fertilization occurred (Fig. 3).

White sucker FS, HS, and SH were excellent in the control and saline water with TDS of 1150 mg/L (Table 2). SH in the control was significantly lower ($P < 0.05$) than in water of 1150 mg/L because of lower fertilization success (Fig. 2). SH in the control and water of 1150 mg/L was significantly greater than water with TDS ≥2400 mg/L. All eggs/embryos were dead within 3 d of fertilization in waters with TDS of 4250 and 6350 mg/L either because of lack of fertilization or development thereafter. Embryo survival in waters with TDS of 200–2400 mg/L was excellent throughout the 11-d incubation period (Fig. 3).

Common carp FS, HS, and SH was 71% in the control and was high (63%) in water with TDS of 4250 mg/L

Fig. 2. Mean survival to hatching of walleye, northern pike, and yellow perch from Devils Lake, white sucker from Many Point Lake, and common carp from the Red River, in waters of 200, 1150, 2400, 4250, and 6350 mg/L total dissolved solids. Vertical bars represent 1 SE. For each species, treatments with different letters are significantly different ($P < 0.05$), Duncan's Multiple Range Test.

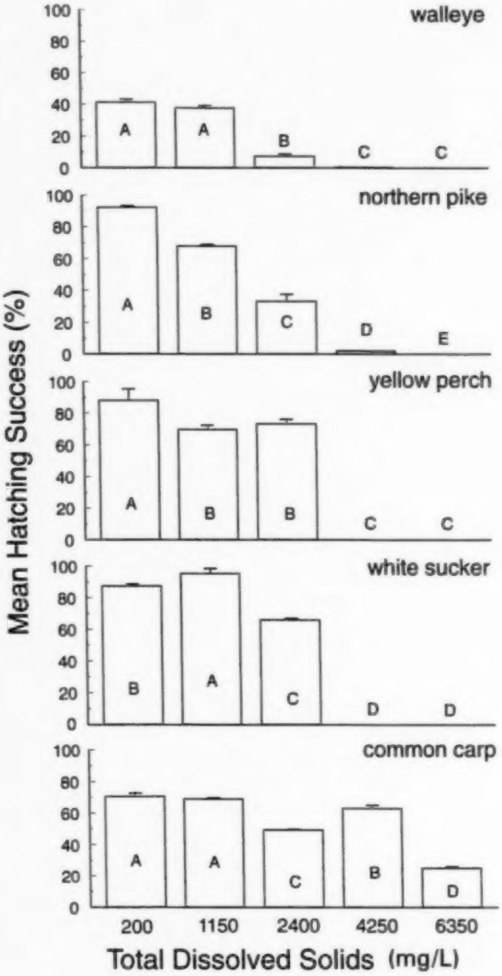
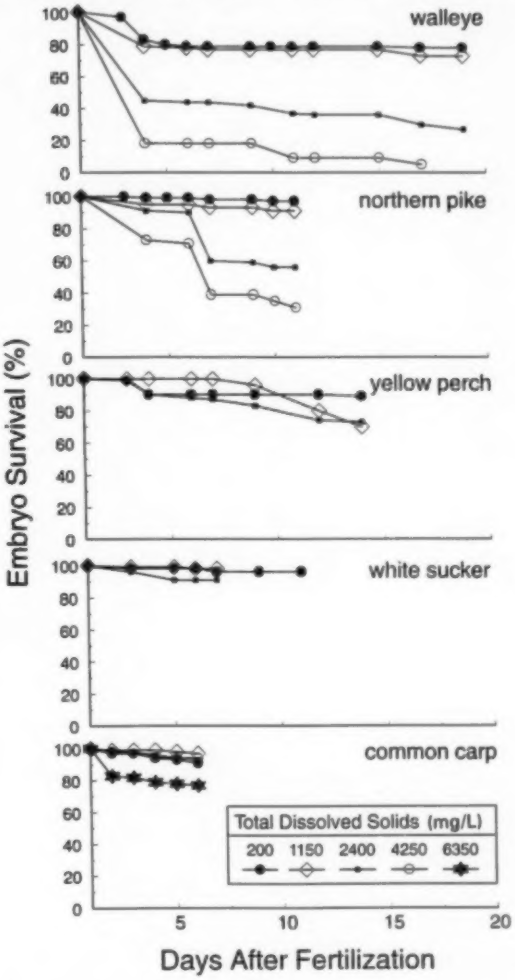


Fig. 3. Percent survival of northern pike, walleye, and yellow perch from Devils Lake, white sucker from Many Point Lake, and common carp from the Red River, from 1 d after fertilization to hatching in waters of 200, 1150, 2400, 4250, and 6350 mg/L total dissolved solids. Survival shown is based on eggs that had been successfully fertilized.



(Table 2). SH in the control and water of 1150 mg/L was significantly greater ($P < 0.05$) than at higher salinities (Fig. 2). Low FS in water of 2400 mg/L was the apparent cause for lower SH in that salinity than in water of 4250 mg/L. Embryo survival was excellent during the 6 d incubation period, except for a period of higher mortality occurring between days 1 and 2 in water of 6350 mg/L (Fig. 3). Common carp was the most tolerant to sulfate salinity of the species studied; HS was 77% in water of 6350 mg/L, but because of the poor FS of only 33%, water with TDS of 6350 mg/L may be near the upper limit tolerated for successful hatching of common carp.

Discussion

The highest salinities where some hatching still occurred was in sulfate-saline water of 4250 mg/L TDS for walleye and northern pike, and 2400 mg/L TDS for yellow perch and white sucker. Based on results of a similar experiment conducted in 1991, the upper limit for SH of yellow perch may be about 4000 mg/L as SH was 91% in water of 4000 mg/L compared with a SH of 97% in the freshwater control.

Atton (1986) reported that white suckers were capable of reproducing in salinities up to 5500 mg/L TDS in sulfate-saline lakes in Saskatchewan; our results indicate that white suckers are much more sensitive to salinity. It is

Table 2. Percent fertilization success (FS),[a] mean percent hatching success (HS), and overall survival to hatching (SH) of eggs obtained from walleye, northern pike, and yellow perch from Devils Lake, white sucker from Many Point Lake, and common carp from the Red River, in waters of 200, 1150, 2400, 4250, and 6350 mg/L total dissolved solids.

	Total dissolved solids (mg/L)				
	200	1150	2400	4250	6350
Walleye					
FS	52.7	51.6	27.3	11.7	2.1
HS	78.1	73.3	27.1	4.5	0.0
SH	41.2	37.8	7.4	0.5	0.0
	(6)	(3)	(3)	(2)	(3)
Northern pike					
FS	95.0	74.6	60.0	5.7	0.7
HS	97.1	91.0	55.5	30.5	0.0
SH	92.2	67.9	33.3	1.7	0.0
	(6)	(3)	(3)	(2)	(3)
Yellow perch					
FS	100.0[b]	100.0[b]	100.0[b]	0.0	0.0
HS	88.1	69.8	73.2	0.0	0.0
SH	88.1	69.8	73.2	0.0	0.0
	(3)	(2)	(2)	(3)	(3)
White sucker					
FS	90.8	98.3	72.5	0.0	0.0
HS	96.3	97.0	91.0	0.0	0.0
SH	87.4	95.4	66.0	0.0	0.0
	(4)	(2)	(4)	(4)	(4)
Common carp					
FS	78.0	71.0	52.9	67.0	32.7
HS	90.7	97.2	93.1	93.9	76.8
SH	70.7	69.0	49.2	62.9	25.1
	(4)	(4)	(4)	(4)	(4)

Note: Number of egg baskets used for each treatment is in parentheses.

[a] Fertilization success was obtained by analyzing at least 100 eggs for each species at each salinity level.

[b] Estimated from embryo survival at 3 d after fertilization.

possible that fishes acclimated to saline waters could have greater reproductive success; white sucker eggs used in our study were obtained from adults living in fresh water. Hatching of white sucker embryos in sulfate-saline waters occurred 4 d sooner than those held in fresh water (Fig. 3). These sac fry were poorly developed, lacked eye pigmentation, and could not swim. It appeared that the chorion deteriorated and became weak, resulting in a premature hatch. However, chorions of eggs held in fresh water remained rigid until hatching occurred, and sac fry were well developed and able to swim.

The common carp was the most tolerant to sodium-sulfate waters of the species tested in this study; hatching occurred in waters as high as 6350 mg/L TDS. Tolerance to high salinities have been noted in other studies. Evermann (1912) found that the common carp was the most abundant fish in the Salton Sea, which had a salinity of 16 000 mg/L (sodium-chloride type water). Kasim (1983) determined that a salinity of 8130 mg/L (sodium-chloride type water) caused only 50% mortality in common carp fingerlings, and in bioassays conducted by Peterka (1992), 90% of common carp embryos successfully hatched in sodium-sulfate type water of 2700 mg/L and 13% hatched in 8450 mg/L. The relatively poorer hatching in sodium-sulfate type water than in sodium-chloride type water probably reflects ionic, rather than osmotic effects.

The SH percentages found in the bioassays didn't account for additional mortality that would be expected to occur in nature. Eggs of walleye, northern pike, white sucker, and carp were soft and lost their adhesiveness in saline waters of 4250 and 6350 mg/L TDS and probably would result in additional mortality of embryos. Both Hendrickson (1990) and Violett (1992) reported the highest catches of northern pike YOY in the Big Coulee drainage where waters were the freshest and where emergent vegetation suitable for spawning northern pike occurred.

Acknowledgments

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Aerial exposure tolerance of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal

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Abstract: We examined the effects of ambient temperature (10, 20, and 30°C) and relative humidity (10, 50, and 95% RH) on the aerial exposure tolerance of adult zebra mussel (*Dreissena polymorpha*) and quagga mussel (*D. bugensis*) collected from the St. Lawrence River. Survivorship of mussels in air significantly increased with increasing RH, decreasing temperature, and increasing mussel size. At 20°C and 50% RH (early temperate summer conditions), large (21–28 mm) *D. polymorpha* survived more than 5 days exposure, whereas small (10–18 mm) *D. polymorpha* survived 1–3 days. Seventy-three percent of large *D. polymorpha* and 10% of small *D. polymorpha* survived 10 days exposure at conditions considered optimal for survivorship (10°C and 95% RH). Survivorship of *D. bugensis* was tested at 20°C and was 15–100% lower than that of *D. polymorpha* at all RH levels combined with exposures less than 5 days. *Dreissena bugensis* also suffered significantly higher percent weight losses because of desiccation than *D. polymorpha*. The differences in the desiccation tolerance of zebra and quagga mussels reflect their relative depth distribution in lakes. Our results suggest that, given temperate summer conditions, adult *Dreissena* may survive overland transport (e.g., on small trailered boats) to any location within 3–5 days' drive of infested waterbodies.

Résumé : Nous avons examiné les effets de la température ambiante (10, 20 et 30°C) et de l'humidité relative, (10, 50 et 95% HR) sur la tolérance à l'exondation de moules zébrées (*Dreissena polymorpha*) et de moules quaggas (*D. bugensis*) adultes prélevées dans le Saint-Laurent. Le taux de survie des moules exposées à l'air augmentait de façon significative en fonction de la croissance de HR, de la baisse de la température et de l'augmentation de la taille des coquillages. À 20°C et 50% HR (conditions tempérées du début de l'été), les *D. polymorpha* de grande taille (21–28 mm) ont survécu à une exondation de plus de cinq jours, tandis que celles de petite taille (10–18 mm) ont survécu de un à trois jours seulement. À des conditions considérées comme optimales pour la survie (10°C et 95% HR), 73% des *D. polymorpha* de grande taille et 10% de celles de petite taille ont survécu à une exondation de dix jours. Le taux de survie de *D. bugensis* a été testé à 20°C et se trouvait de 15–100% inférieur à celui de *D. polymorpha* à tous les niveaux de HR combinés à des exondations de moins de cinq jours. Les *Dreissena bugensis* ont aussi subi des pertes de poids en pourcentage, dues à la déshydratation, nettement supérieures à celles de *D. polymorpha*. Les différences dans la tolérance à la déshydratation chez les moules zébrées et quaggas reflètent leurs distributions relatives en profondeur dans les lacs. Nos résultats permettent de penser que, dans des conditions estivales tempérées, les *Dreissena* adultes peuvent survivre au transport terrestre (par exemple si elles sont fixées à la coque de petits bateaux transportés par remorque) en n'importe quel point situé à 3–5 jours de distance des plans d'eau infestés.
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Introduction

Since its initial introduction in Lake St. Clair ca. 1986 (Hebert et al. 1989), the exotic zebra mussel (*Dreissena polymorpha*) has spread rapidly throughout the Great Lakes and into several major river systems (e.g., the St. Lawrence, Hudson, Ohio, Mississippi, and Tennessee rivers) and has caused substantial economic and ecological impacts (e.g., Schloesser and Kovalak 1991; Kovalak et al. 1993; Lepage 1993; MacIsaac et al. 1993; Holland 1993). Its rapid expansion has been linked to its possession of planktonic veliger larvae, byssal threads (for attachment to hard surfaces), and high rates of growth and recruitment (Stanczykowska 1977; Carlton 1993). A second dreissenid recently found in the Great Lakes, the quagga mussel (*Dreissena bugensis*), is also expanding its range (Mills et al. 1993) and may have negative impacts on profundal fauna (Dermott and Munawar 1993).

Because *Dreissena* can potentially colonize most of the major lakes and rivers on the continent (Strayer 1991; Ramcharan et al. 1992), it is important to determine the factors that allow the mussels to spread into new habitats. The principal downstream dispersal vector in river systems is larval drift (Griffiths et al. 1991), but humans may spread zebra mussels considerable distances upstream on the hulls of commercial barges (Keevin et al. 1992) and to isolated lakes and rivers through fishing and boating activity (Carlton 1993; McNabb 1993). The potential for overland transport of byssally attached mussels on trailored boats has also been strongly emphasized (Griffiths et al. 1991; Neary and Leach 1992; Johnson and Carlton 1993; Carlton 1993). Dreissenid mussels may attach to boat hulls or to aquatic vegetation that is caught on boating equipment and trailers; up to 25% of recreational boat trailers departing certain Michigan boat ramps are estimated to be carrying adult zebra mussels (Johnson and Carlton 1993). The movement of mussel-fouled chains, fishing nets, buoys, boat docks, and other fishing and boating equipment between water basins may also help disperse dreissenids (Carlton 1993). The successful overland transport of dreissenid mussels by these vectors will depend primarily on their ability to tolerate periods of aerial exposure (desiccation).

References to the desiccation tolerance of *Dreissena* in the literature are generally anecdotal and lack quantitative information on air temperature or relative humidity conditions. Some authors refer vaguely to the ability of *Dreissena* to tolerate aerial exposure for periods of "a few days" (O'Neill 1990) to "several days" (Neary and Leach 1992). Mussels attached to a car pulled out of Lake Erie survived 4 days in air (Griffiths et al. 1991). In a laboratory study, Alyakrinskaya (1978) observed that *D. polymorpha* remained alive out of water for 4 days at temperatures of 20–22°C and variable relative humidity. Wisniewski (1992) noted that 96% of *D. polymorpha* placed on moist sand in a Polish reservoir survived 3 days exposure to air. Conversely, *D. polymorpha* survived 14 days in the cool, moist interior of an unused water pipe (Hoestland and Lassabliere 1959). McMahon and Paine (1992) found that both relative humidity and temperature had a significant effect on the survivorship of emersed mussels. No previous study has assessed the effects of mussel size on the aerial exposure tolerance of *D. polymorpha*, and there are no published data on the aerial exposure tolerance of *D. bugensis*. This information would help predict the rate of overland spread of *Dreissena* and aid in the development of methodologies to control mussel dispersal.

We examined the effects of temperature, relative humidity, exposure period, and mussel size on the survivorship of *D. polymorpha* and *D. bugensis* in air. Given that *D. bugensis* is typically found at greater depths in lakes than *D. polymorpha* (Mills et al. 1993), we hypothesized that it is less adapted (i.e., less tolerant) to aerial exposure.

Methods

Dreissenid mussels (*D. polymorpha* and *D. bugensis*) were collected in the summer and autumn of 1992 by SCUBA at Soulanges Canal, a section of the St. Lawrence River southwest of the Island of Montreal (45°20′N, 73°58′W); the mean summer density and biomass of dreissenids at this site were 1990 mussels/m^2 and 1568 g (fresh wt., including shells)/m^2, respectively (A. Ricciardi, unpublished data). *Dreissena bugensis* (distinguished by the convex ventral margin of its shell) occurred in the collections in small numbers (1 of 100 dreissenids) and were separated from *D. polymorpha*. These collections were used to establish laboratory colonies for use in our experiments.

Prior to experimentation, the mussels were kept in aerated aquaria at 20°C and fed daily with dried *Chlorella* or with phytoplankton produced in separate aquaria. To determine if smaller *D. polymorpha* desiccate at a faster rate than larger mussels, two size classes (SC) were used: 10–18 mm (SC1: mean length ± SE = 15.0 ± 1.4 mm) and 21–28 mm (SC2: mean length ± SE = 24.0 ± 1.2 mm); in the upper St. Lawrence River in summer, these size classes generally correspond to 1- and 2-year old *D. polymorpha*, respectively (determined by size–frequency distributions; A. Ricciardi, unpublished data). Groups of 10 live mussels belonging to one size class were placed in desiccating chambers (glass jars approximately 3 L in volume, with screw-on lids) and subjected to treatment combinations of three temperatures (10, 20, 30°C), three relative humidities (RH = 10, 50, 95%), and three exposure periods (1, 3, 5 days). The exposure periods were chosen based on reports of zebra mussel tolerance to drying in situ. Temperatures and relative humidities were chosen to represent ranges comparable to those experienced in the St. Lawrence River valley during the ice-free periods of the year. The 10°C treatments were conducted in an incubator (Sherer model CEL255-6); 20°C treatments were conducted in the laboratory (controlled ambient air temperature of 19–21°C); 30°C treatments were conducted in a heated circulating water bath. Graded solutions of sodium hydroxide were prepared as desiccants, following Madge (1961). Individual mussels were removed from their aquaria, blotted dry on tissue paper, numbered with a permanent marker, measured for maximum length (using callipers), and weighed (wet weight, i.e., shell + meat + internal water) before each treatment. The mussels were then placed in an uncovered Petri dish which was elevated at least 3 cm above the desiccating solution by plastic supports. A relative humidity of approximately 95% was obtained by using distilled water

Table 1. Mean percent survivorship of *D. polymorpha* in air at three different exposures (1, 3, 5 days) of combinations of three levels of relative humidity (10, 50, 95%) and ambient temperature (10, 20, 30°C).

	Mussel length, 10.0–18.0 mm									Mussel length, 21.0–28.0 mm								
	10°C			20°C			30°C			10°C			20°C			30°C		
%RH	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d
10	93.3	26.7	0*	66.7*	3.3	0	0*	0	0	93.3	36.7	25.0*	96.7*	13.3	0	60.0*	0	0
	(6.7)	(16.7)	(0)	(12.0)	(3.3)	(0)	(0)	(0)	(0)	(3.3)	(8.8)	(5.0)	(3.3)	(6.7)	(0)	(0)	(0)	(0)
50	93.3	90.0	3.3**	72.5*	3.3**	0	0**	0	0	100	100	100**	96.7*	73.3**	16.7	77.5**	0	0
	(6.7)	(0)	(3.3)	(7.5)	(3.3)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(3.3)	(13.3)	(12.0)	(8.5)	(0)	(0)
95	96.7	90.0	73.3*	96.7	70.0*	13.3*	33.3*	0	0	100	100	100*	100	100*	47.5*	100*	3.3	0
	(3.3)	(5.8)	(6.7)	(3.3)	(0)	(8.8)	(17.6)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(11.1)	(0)	(3.3)	(0)

Note: Standard errors are in parentheses. Asterisks indicate significant differences between size classes: *, $p < 0.05$; **, $p < 0.01$.

Table 2. Mean percent survivorship of dreissenid mussels after prolonged (10, 15 d) exposure in air in cool, moist (10°C, 95% RH) conditions.

Size class	Exposure (days)	PWL	% survivorship
	D. polymorpha		
I	10	41.2 (1.2)	6.7 (3.3)
II	10	12.5 (1.3)	73.3 (12.0)
I	15	44.7 (0.1)	0 (0)
II	15	36.5 (0.5)	0 (0)
	D. bugensis		
I	10	44.0	0
II	10	17.6	40.0
II	15	43.0	0

Note: Except for *D. bugensis*, all values are averages of three replicates; standard errors are in parentheses. PWL, percent weight loss.

alone. Relative humidity levels were monitored daily by inserting a hygrometer probe (Climomaster model 6511) into the desiccating chamber, and blocking the opening with a plastic bag while reading the hygrometer. Each chamber was opened briefly each day to check the relative humidity and to make necessary adjustments by adding either NaOH solution or distilled water. Average daily temperature and relative humidity fluctuations were ±2.0°C and ±5%, respectively.

The treatment chambers were sampled by a gas chromatograph to determine if oxygen was in sufficient supply. Five-millilitre samples of air were taken at the beginning of a trial and again 24 h later. The air samples were analyzed using a Fischer-Hamilton model-28 gas partitioner, which indicated that the oxygen concentration within the chambers (both before and after 24 h) was not significantly different from the atmospheric concentration. Air was replenished in the chambers every 24 h when they were opened briefly to check relative humidity levels.

Each treatment was replicated two to four times. Controls consisted of mussels (SC1) immersed in aerated, plankton-free water at 10, 20, and 30°C, for 1-, 3-, and 5-day exposures and were replicated three times. At the end of each treatment, mussels were reweighed, and their viability determined by 24 h reimmersion in a 1-L flask of aerated water maintained at 20°C and containing phytoplankton. If, after 24 h, the mussel did not extend its siphon, move its valves, lay down byssi, or respond to prodding, it was considered dead. During treatment inspections, mussels that showed obvious signs of death (e.g., extended shell gape) were immediately removed from the desiccating chamber.

The same protocol was used to determine the aerial exposure tolerances of *D. bugensis*; however, fewer trials were run, because of the scarcity of quaggas in our collections. Quaggas were tested at each relative humidity – exposure period combination at 20°C. We used specimens belonging to one size class with a length range of 12.0–18.0 mm (mean ± SE = 16.0 ± 1.1 mm).

In a separate set of experiments using similar protocols, the prolonged aerial exposure tolerance of both size classes

Table 3. Mean percent survivorship and percent weight loss of *D. bugensis* in air at three different exposures (1, 3, 5 days) and relative humidities (10, 50, 95% RH) at 20°C.

	% survivorship			% weight loss		
%RH	1 d	3 d	5 d	1 d	3 d	5 d
10	35.0	0	0	43.5	62.3	62.0
	(15.0)	(0)	(0)	(0.3)	(0.1)	(1.9)
50	40.0	0	0	42.7	59.0	63.5
	(5.8)	(0)	(0)	(2.4)	(1.8)	(1.2)
95	85.0	10.0	0	8.3	15.7	41.7
	(15.0)	(10.0)	(0)	(0.2)	(0)	(6.7)

Note: Standard errors are in parentheses.

of *D. polymorpha* was determined for 10 and 15 days exposure in optimal conditions (10°C, 95% RH); three replicates were run for each exposure period. The survivorship of SC2 *D. polymorpha* exposed for 10 days at 20°C and 95% RH was also tested (three replicates). The survivorship of *D. bugensis* in prolonged (10 and 15 days) exposure was tested at 10°C and 95% RH for two size classes (12.0–18.0 and 21.0–24.0 mm); because of the insufficient number of available mussels, these experiments could not be replicated.

Results were analyzed by ANOVA (General Linear Models procedure; SAS Institute Inc. 1988), and Bonferroni-adjusted *t*-tests were used in multiple comparisons of treatment effects.

Results

Survivorship of *D. polymorpha* in air

Temperature, RH, and exposure period explained 95% of the observed variance in survivorship of small (SC1) *D. polymorpha* (ANOVA, $p < 0.0001$, df = 79). At 10°C, 100% mortality occurred within 3–5 days at 10% RH and within 5–10 days at 50–95% RH (Table 1). At 20°C, 100% mortality occurred within 3–5 days at 10–50% RH and 5–6 days at 95% RH. At 30°C, 100% mortality occurred in less than 24 h at 10–50% RH and within 1–3 days at 95% RH. The mean percent survivorship after 10 days exposure at 10°C and 95% RH was 6.7%; no mussel survived 15 days exposure (Table 2).

Temperature, RH, and exposure period explained 96% of the observed variance in survivorship of large (SC2) *D. polymorpha* (ANOVA, $p < 0.0001$, df = 78). At 10°C, 100% mortality occurred within 5–10 days at 10–50% RH and within 10–15 days at 95% RH (Tables 1 and 2). At 20°C, 100% mortality occurred within 3–5 days at 10% RH and 5–7 days at 50–95% RH. At 30°C, 100% mortality occurred within 1–3 days at 10–50% RH and within 3–5 days at 95% RH. Survivorship of SC2 *D. polymorpha* at 10°C and 95% RH was 73% after 10 days but declined sharply thereafter to reach 0% at 15 days (Table 2). Although 21% of SC2 mussels survived 5 days exposure at 20°C and 95% RH, all mussels died within 10 days of prolonged exposure in these conditions.

Table 4. Mean percent weight loss of *D. polymorpha* in air at all desiccation treatments.

	Mussel length, 10.0–18.0 mm									Mussel length, 21.0–28.0 mm								
	10°C			20°C			30°C			10°C			20°C			30°C		
%RH	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d	1 d	3 d	5 d
10	22.7**	42.7*	51.2	32.9**	58.2**	62.0*	39.4**	60.2	60.3	12.2**	34.0*	41.7	20.4**	43.8**	55.7*	29.7**	50.8	60.2
	(1.8)	(1.6)	(5.5)	(1.2)	(0.2)	(0.9)	(0.5)	(0.4)	(0.8)	(0.4)	(2.5)	(0.7)	(0.5)	(0.6)	(1.6)	(2.0)	(3.8)	(1.1)
50	11.5*	24.6	46.1*	21.2*	48.5**	61.4**	40.0**	60.6	63.8	8.4*	16.7	29.3*	12.2*	28.2**	35.4**	25.8**	55.0	57.0
	(0.8)	(3.1)	(6.9)	(2.4)	(3.9)	(1.1)	(2.3)	(0.3)	(0.6)	(0.5)	(0.2)	(1.8)	(0.8)	(0.9)	(1.4)	(1.3)	(3.2)	(2.2)
95	4.9	6.9	11.6	6.6	13.7	19.1	21.5	52.1	61.4*	4.3	5.1	9.8	5.4	9.1	20.9	14.2	40.6	46.3*
	(1.1)	(1.0)	(1.8)	(1.8)	(1.8)	(1.3)	(4.8)	(1.0)	(1.6)	(0.7)	(0.8)	(0.4)	(1.2)	(1.4)	(3.4)	(1.3)	(9.8)	(3.4)

Note: Standard errors are in parentheses. Asterisks indicate significant differences between size classes: *, $p < 0.05$; **, $p < 0.01$.

Overall, experimental treatment (temperature, RH, exposure period, mussel size) explained 86% of variation in survivorship ($p < 0.0001$) of *D. polymorpha* when both size classes were combined.

Survivorship of *D. bugensis* in air

Relative humidity and exposure period explained 92% of the observed variation in survivorship of *D. bugensis* (12–18 mm shell length) at 20°C (ANOVA, $p < 0.0001$, df = 20). Survivorship of *D. bugensis* for 24 h in air was similar at 10 and 50% RH; all mussels died within 3 days (Table 3). At 95% RH, only 10% of *D. bugensis* survived 3 days exposure (none survived 5 days), contrasting with the high survivorship (70–100%) of *D. polymorpha* over the same period. Under cool, humid conditions (10°C, 95% RH), large *D. bugensis* (21–24 mm shell length) survived at least 10 days aerial exposure but with lower survivorships than large zebra mussels (Table 3). Mean survivorship of small *D. bugensis* (12–18 mm shell length) was compared with that of small *D. polymorpha* (SC1) at 20°C using *t*-tests and was found to be lower ($p < 0.05$) at all RH combined with exposures less than 5 days.

Weight loss of zebra and quagga mussels because of desiccation

In general, the percent weight loss (PWL) of dreissenids because of desiccation was directly proportional to temperature and exposure period, and inversely proportional to RH (Tables 3 and 4). Experimental treatments explained 94 and 97% of the variation in the PWL of *D. polymorpha* and *D. bugensis*, respectively. However, a wide range of mean weight loss was observed for *D. polymorpha* at 0% mortality (3.6–30.9 PWL; mean = 12.3 ± 1.2) and 100% mortality (16.5–64.4 PWL; mean = 53.4 ± 1.3). No individual zebra mussel survived a weight loss greater than 48%.

Dreissena bugensis suffered higher PWLs than SC1 and SC2 *D. polymorpha*, at treatment combinations of 20°C, all RH, and exposures less than 5 days (multiple *t*-tests, $p < 0.05$). PWLs of *D. bugensis* at different exposures were not significantly different at 10% and 50% RH (Table 3).

Discussion

Our results suggest that the successful overland dispersal of byssally attached dreissenid mussels exposed to air for a short period of time (days) is highly probable. We found that large *D. polymorpha* (length >20 mm) may survive 5 days in still air in environmental conditions typical of an early temperate summer (20°C, 50% RH). This compares favourably with reports of *D. polymorpha* surviving 3–4 days emersion in natural (and variable) conditions (Griffiths et al. 1991; Wisniewski 1992). Mussels may survive 10–15 days out of water in the cool, humid conditions (10°C, 95% RH) that often occur in late autumn or early spring (Table 2).

Despite different methodologies (desiccation procedure, temperature, and RH regimes), the tolerance times observed for *D. polymorpha* in our study were similar to those reported by McMahon and Paine (1992). McMahon and Paine (1992) found that 100% mortality of *D. polymorpha* (size range not specified) occurred between 15 and 20 days at low temperature (5°C) over a RH range of 5–75%. By contrast, no mussels in our experiments survived 15 days exposure at a combination of low temperature (10°C) and high RH (>95%), but differences in acclimation and treatment temperatures may account for this disparity.

Causes of mortality

Iwanyzki and McCauley (1993) found that 10–20 mm *D. polymorpha* (corresponding approximately to our SC1 length range) that were acclimated at 20°C survived less than 3 days in water at 30°C, and survivorship rapidly declined with every degree increase above 30°C. None of our SC1 *D. polymorpha* (also acclimated at 20°C) survived 24 h aerial exposure at 30°C and RH ≤ 50%, indicating that mortality was not due to thermal stress alone. The small percentage of SC2 mussels that survived more than 3 days exposure at 30°C and 95% RH suggests that desiccation resistance and thermal tolerance may increase with mussel size. Smaller *D. polymorpha* have a higher tissue surface area to volume ratio and thinner shells (with possibly higher permeability), and therefore may lose water more rapidly when desiccated (cf. Schaefer et al. 1968; Byrne et al. 1988; Thivakaran and Kasinathan 1990). Differences in survivorship between the zebra mussel size classes were most pronounced at 20°C (Table 1), while differences in PWL were most pronounced at temperatures of 10–20°C and 10–50% RH. Differences in PWL tended not to be significant at 95% RH.

The observed change in mussel weight over the treatment period was assumed to represent a combination of (*i*) evaporative water loss (tissue water + water in the mantle cavity) due to desiccation and (*ii*) resorption of energy stores because of starvation and stress. The difference in mussel weight loss at low (10% RH) and high (95% RH) relative humidities became more pronounced at lower temperatures; at 10°C, the PWL at 10% RH was three to six times greater than at 95% RH, whereas at 30°C, it was generally one-half to three-quarters that at 95% RH (Table 4). In our experiments, groups of mussels that experienced similar weight losses did not always suffer similar mortalities, suggesting that evaporative (water) loss because of desiccation was not the sole cause of mortality. Other causes may include depletion of energy reserves, acidosis, toxic buildup of anaerobic metabolites, or a combination of these factors (Byrne et al. 1990). Alyakrinskaya (1978) found that *Dreissena* buffered a biochemically driven shift in acidity during desiccation by releasing calcium ions through shell dissolution, but it is not known whether this mechanism would be effective during prolonged aerial exposure. In our study, *D. polymorpha* and (to a lesser extent) *D. bugensis* were observed to periodically gape (i.e., partially open their valves and expose their mantle tissue) during aerial exposures at high RH (=50–95%), but rarely at low RH (=10%); gaping behaviour for *D. polymorpha* was also noted by McMahon and Paine (1992). This behaviour suggests that the mussels were maintaining an aerobic metabolism that may delay the lethal buildup of anaerobic endproducts during emersion; however, metabolites such as ammonia may accumulate to toxic levels during prolonged aerial exposure (as in *Corbicula*, cf. Byrne et al. 1990).

Effects of microhabitat selection and mussel clustering on aerial exposure tolerance

Mussel survival may increase within a cluster, which would retain water vapour, reduce exposed surface area, and provide shelter from convective air currents. Multilayered *D. polymorpha* clusters are common wherever populations have had adequate time to establish high densities (Wiktor 1963; Morton 1969; Stanczykowska 1977; Hebert et al. 1989). The tendency for dreissenids to aggregate along seams and in crevices (Mellina and Rasmussen 1994; A. Ricciardi, personal observation), where they retain moisture and are sheltered from direct sunlight and wind, should enhance their aerial exposure tolerance. In the St. Lawrence River estuary, mussel colonies occur in crevices on the surface of boulders exposed to direct sunlight at low tide (Mellina and Rasmussen 1994). The microclimate within these colonies may retain moisture and expose internal occupants to higher humidity levels. Conversely, this benefit may be offset by adverse conditions created by densely packed multilayered clusters, e.g., lower oxygen tension and higher ammonia levels; a dense cluster may trap and accumulate ammonia, and mussels byssally bound by their neighbours may be prevented from effectively gaping.

Griffiths et al. (1991) noted that densely packed mussels attached to the exterior of a car pulled out of Lake Erie survived 4 days in summer weather, but he did not mention the proportion of surviving mussels. For short periods of time, the benefits of clumping probably outweigh the costs; differences in the aerial exposure tolerance for a monolayered bed of mussels (very common in nature where substrate is not limiting; A. Ricciardi, personal observation) versus a multilayered cluster may be important. The monolayer may retain water within its interstices, and perhaps increase boundary-layer resistance to desiccation by convection, but probably would not suffer the adverse effects associated with multilayered clumping. Therefore, we expect that a small aggregation, or monolayer, of mussels would survive longer periods of overland dispersal than mussels in multilayered clumps.

Individual mussels within clumps of vegetation may also be protected against enhanced desiccation from sunlight and wind. *Dreissena* attaches to vegetation in lakes (Lewandowski 1982; UW Sea Grant Institute 1993), and vegetation is commonly transported between lakes on boating equipment (e.g., Johnstone et al. 1985). Furthermore, *D. polymorpha* has been found on aquatic weeds caught on boat trailers departing from the Great Lakes, and many boaters travel between the Great Lakes and inland waterbodies on a daily or weekly basis (Johnson and Carlton 1993; Gunderson 1994); successful interlake dispersal is highly probable over these short time periods (Table 1).

Mitigation strategies based on aerial exposure

Dreissena polymorpha is a common fouling organism on the walls of reservoirs (Oldham 1930; Morton 1969; Wisniewski 1992) and may be periodically exposed by drawdown. From our data, 100% mortality of mussels on reservoir walls could be achieved by drawdown for 5–10 days at temperatures greater than 20°C. Because *D. polymorpha* occurs in greatest densities at depths of 2–4 m (Stanczykowska 1977), controlled drawdown of reservoirs in midsummer could eradicate the majority of the mussels, and confine the remaining mussels to unfavorable habitats (e.g., anoxic hypolimnia). This method has been advocated as a control for another invader of reservoirs, the Asiatic clam *Corbicula fluminea* (White 1977; Byrne et al. 1988). For mussels infesting the cool interior of a water pipe, 100% mortality would require at least 15 days after dewatering (assuming >95% RH), but desiccation could be accelerated by convective drying with heated air (cf. Jenner and Janssen-Mommen 1993).

Aerial exposure tolerance as a factor governing the depth distribution of dreissenids

Dreissena polymorpha's tolerance of short periods of emersion may be an important factor governing its ability to colonize hard substrate in shallow waters. Zebra mussels are most abundant in the littoral and sublittoral zones of lakes and reservoirs (Stanczykowska 1977), and also occur in intertidal areas (Strayer and Smith 1993; Mellina and Rasmussen 1994) where they must be endure periodic exposure to the air and sun. By contrast, *D. bugensis* is the dominant dreissenid in deep waters in the Laurentian Great Lakes (up to 130 m, cf. Mills et al. 1993) and in profundal areas of reservoirs along the Dnieper River in the Ukraine (Pligin 1984), and therefore might be expected to be less adapted to desiccation. The lower aerial exposure tolerance of *D. bugensis* observed in our experiments supports this hypothesis. The patterns we observed are analogous to those of intertidal marine bivalves and other invertebrates whose depth distribution is inversely correlated with their desiccation resistance (Kensler 1967; Landenberger 1969; Foster 1971). Ussery and McMahon (1994), working independently from us, recently compared the desiccation tolerance of *D. polymorpha* and *D. bugensis* at 15°C under a variety of RH and found that *D. bugensis* suffered greater mortality than *D. polymorpha* at >50% RH; these results lend further support to the contention that the deeper water dreissenid is less likely to survive prolonged aerial exposure during overland dispersal.

Conclusions

The factors contributing to the successful establishment of exotic species in new habitats have not been fully elucidated (Elton 1958; Lodge 1993). If invasion success depends on the size of the introduced (pioneer) population, then the overland transport of highly fecund adults may have greater invasion potential than the transport of larvae across short geographic distances. Since many recreational boaters use the Great Lakes and smaller inland lakes within the same week (Johnson and Carlton 1993; Gunderson 1994), the successful overland dispersal of adult dreissenid mussels from established Great Lakes populations is highly probable. Our results indicate that, given average summer weather conditions, *D. polymorpha* could be transported overland to any location within a radius of 5 days' drive of infested areas. There have been sightings of adult dreissenids in water bodies isolated from the Great Lakes system and which required overland transport for colonization; these include several inland lakes in Michigan, Indiana, Ohio, and New York (Marangelo and Johnson 1993; University of

Wisconsin Sea Grant Institute 1994). There is also evidence that *Dreissena* was introduced into the Hudson River at Catskill, New York State, by humans rather than by larval drift from the Erie Canal (Strayer and Powell 1992); this may have been accomplished by an infested boat. The probability of an isolated lake being colonized by dreissenid mussels transported overland on boat trailers should increase substantially as the distance between the mussel's range and the lake decreases, enhancing the conditions for successful overland dispersal. Water bodies in close proximity to major highways (allowing greater traffic between invaded and uninvaded sites) should be considered particularly vulnerable to invasion by overland dispersal.

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Growth and condition in the Northwest Atlantic harp seal

M.O. Hammill, M.C.S. Kingsley, G.G. Beck, and T.G. Smith

Abstract: We analysed the relationship of length to age in a sample of 204 harp seals collected in 1976–1979 and of 437 animals collected in 1988–1992, by fitting asymptotic growth curves. The only significant variations were in asymptotic length, which for 1988–1992 was significantly larger for males (170.4 cm) than for females (165.3 cm). This sexual dimorphism was not significant in 1976–1979, because the fitted asymptotic length of females varied greatly from year to year, being large in the 1976–1979 samples. An index of body condition (total weight/length$^{2.6}$) and a volume index constructed by regressing body weight on length × axillary girth squared indicated that harp seals collected in 1988–1992 were in poorer condition than animals collected during 1976–1979. Although this suggests a decline in available resources, the possibility of differences arising from sampling bias cannot be excluded.

Résumé : Nous avons analysé la relation entre la longueur et l'âge de 204 phoques du Groenland recueillis en 1976–1979 et de 437 autres recueillis en 1988–1992, en ajustant des courbes de croissance asymptotique. Les seules différences significatives provenèrent de la longueur asymptotique, qui en 1988–1992, était significativement plus grande pour les mâles (170,4 cm) que pour les femelles (165,3 cm). Ce dimorphisme sexuel n'etait pas significatif en 1976–1979, car la longueur aymptotique ajustée des femelles variait beaucoup d'une année à l'autre, étant plus grande pour les échantillons de 1976–1979. Un indice de condition physique (poids total/longueur$^{2.6)}$ et un indice de volume établi à partir de la relation entre poids total et longueur circonférence axillaire^2, indiquent que les phoques du Groenland recueillis en 1988–1992 étaient en moins bonne condition que ceux recueillis dans les années 1970. Bien que ceci suggère une réduction des ressources disponibles, on ne peut exclure la possibilité que les différences proviennent de biais d'échantillonnage.

Introduction

Changes in the relation of population size to available resources may result in changes in physical condition and growth of individuals. This could subsequently affect population parameters such as age at sexual maturity, reproductive rates, and survival rates (Laws 1956, 1959; Bowen et al. 1981; Eberhardt and Siniff 1977; Frisch 1978; McLaren and Smith 1985). Also, because energy requirements are linked to body size (Kleiber 1975), changes in growth will affect quantitative models of population energy requirements (Lavigne et al. 1982, 1985; Markussen et al. 1989).

In marine mammals, the level of fat reserves, stored principally as blubber, has been considered to be the best indication of body condition (Beck et al. 1993*a*). Condition has been assessed using some index of fat stores such as body weight, sculp weight, axillary girth, maximum girth, or a blubber thickness corrected for body size using length or length squared (Beck et al. 1993*a*).

Growth of mammals has frequently been summarized by fitting nonlinear models such as the logistic, Gompertz or von Bertalanffy models to length-at-age data (Turner et al. 1976; Zullinger et al. 1984; McLaren and Smith 1985; Appendix 1). The subsequent evaluation of growth curve parameters permits a general and flexible means for comparing different populations and for comparing the same population at different times (Kingsley 1979) or under different conditions (Eberhardt and Siniff 1977; Zach and Mayoh 1984).

The harp seal (*Phoca groenlandica*) is a migratory, ice associated, north Atlantic seal. The Northwest Atlantic population whelps in late February and March off the east coast of Newfoundland (Front) and in the Gulf of St. Lawrence (Gulf). During the summer harp seals occupy Arctic regions as far north as Jones Sound. Hunted commercially since the 18th century, the population is believed to have declined by 52% between 1952 and 1968 (Sergeant 1991). In 1972, a harvest quota was introduced that was intended to allow the herd to increase. Since the early 1980s, with the end of the commercial hunt from large ships, harvests have declined from an average of 172 000 per year between 1978 and 1982 to less than 50 000 per year between 1983 and 1990 (Anonymous 1991). Currently, the Northwest Atlantic harp seal population is thought to be expanding (Stenson et al. 1993).

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Table 1. Number of harp seals, location of sample collection, and date of collection.

Location	Date of collection	Number
Magdalen Islands (Gulf)	March 1976	25
	March 1977	11
	March 1978	24
	April 1978	23
	March 1979	26
Newfoundland (Front)	April 1976	52
West Greenland	Aug.–Sept. 1976	5
Northwest Territories	July–Aug. 1977	37
Les Escoumins (St. Lawrence estuary)	Dec. 1988 – Feb. 1989	40
	Apr. 1989	10
	Dec. 1991 – Mar. 1992	104
Magdalen Islands (Gulf)	March 1989	171
	Feb.–Mar. 1990	49
	Mar. 1991	18
	Mar. 1992	36
Hudson Strait	Sept.–Oct. 1989	11
Harrington Harbour (N. Gulf of St. Lawrence)	Dec. 1989	17

Note: Samples collected prior to 1980 are from Innes et al. 1981. New samples were obtained after 1988.

Table 2. Number of males and females, mean age, and standard errors for males and females collected between 1976–1979 and 1989–1992.

Year	Sex	*N*	Mean age	SE	Maximum age	*K*
1976	F	48	8.5	1.1	25	2.3*
	M	34	2.4	0.6	14	
1977	F	21	8.9	1.9	25	0.7
	M	27	11.2	1.7	29	
1978	F	34	11.4	1.4	28	2.2*
	M	13	1.6	0.6	9	
1979	F	24	11.8	1.3	26	0.6
	M	2	12.5	2.5	15	
1988	F	5	11.6	5.0	31	0.6
	M	12	11.7	1.8	24	
1989	F	98	11.7	0.6	26	0.9
	M	134	10.6	0.5	27	
1990	F	18	9.7	1.0	21	0.8
	M	12	11.4	1.2	20	
1991	F	6	4.7	2.1	14	0.4
	M	18	4.4	1.1	19	
1992	F	29	7.7	1.2	24	0.4
	M	61	7.2	0.9	28	

Note: Age distributions were compared between sexes using a Kolmogorov–Smirnov two-sample test (Siegel 1956). *K* is the Kolmogorov–Smirnov statistic (SAS Institute 1987). *, denotes significant differences.

Coincident with the apparent increase in the harp seal population, there has been a decline in the Atlantic northern cod stocks. Reasons for this decline have not been well defined, but it has been suggestd that the role played by harp seals in cod–capelin predator–prey relationships should be examined (Harris 1990).

These apparent changes in harp seal abundance and availability of food resources since the early 1980s may have had an effect on harp seal growth and condition. Innes et al. (1981) examined relationships between age, length, and weight in harp seals from the Northwest Atlantic and concluded that neonate harp seals sampled in the 1970s were heavier and larger than animals harvested during the 1950s. Their data also indicated that harp seals were monomorphic.

Here, we compare growth and condition in harp seals collected between 1988 and 1992 with an earlier sample of harp seals collected during 1976–1979 (Innes et al. 1981).

Materials and methods

Morphometric data were collected from 437 harp seals taken in the Gulf of St. Lawrence, the St. Lawrence Estuary, and Hudson Strait between 1988 and 1992 (Table 1). All animals were shot in the water and retrieved either using a small boat or from the ice edge. Standard length and axillary girth (American Society of Mammalogists 1967) were measured to the nearest centimetre. Body weight was determined to the nearest 0.5 kg using a dial scale suspended from a tripod. Age was determined by counting incremental growth layers in the dentine of transverse sections cut from a lower canine tooth (Bowen et al. 1983).

Age–length relationships were also fitted to data for a sample of 204 harp seals collected during 1976–1979 (Innes et al. 1981) and compared with the more recent sample.

Seals in the current study were assigned ages to the nearest day assuming they were born on 2 March (Stewart et al. 1989). Growth was described using a Gompertz growth curve (Sugden et al. 1981):

$$l_t = L_\infty \exp\{-\exp[-ek(t - t_{\text{zero}})]\}$$

where l is length (cm) at age t, L is asymptotic length (cm), k is the maximum relative growth rate (year^{-1}), and t_{zero} is age at the maximum rate of growth (Sugden et al. 1981). t_{zero} often appears as a negative value and has little

Fig. 1. Length at age for male and female harp seals collected during 1988–1992.

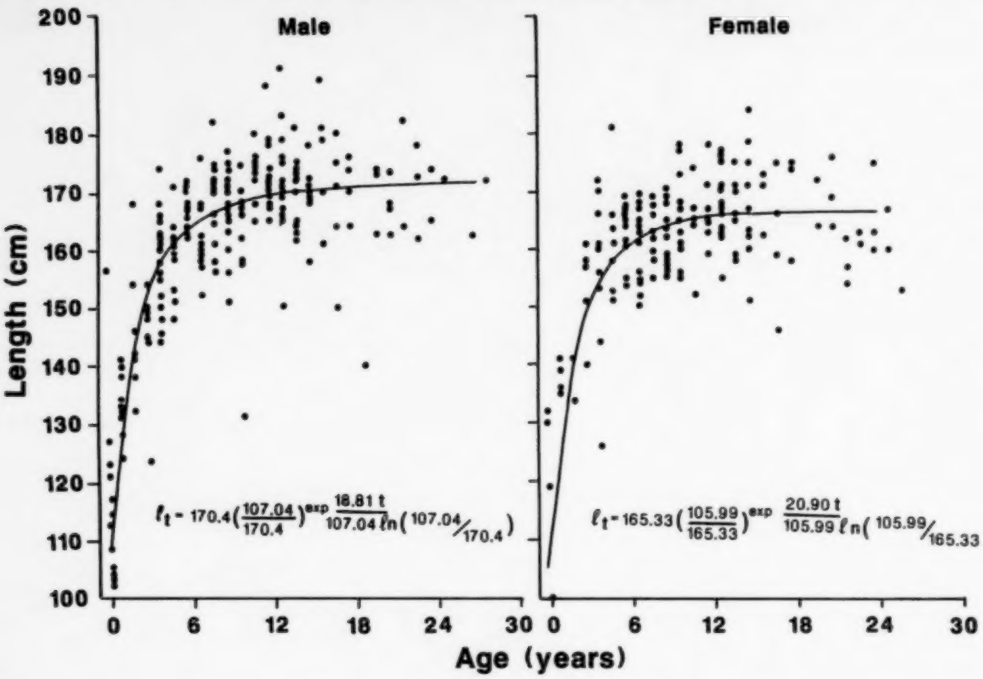


Table 3. Parameters of Gompertz growth curves fitted to Northwest Atlantic harp seal age-at-length data.

Sample	Sex	*N*	Asymptotic length (cm)	Estimated length at birth (cm)	Growth rate (cm·year^{-1})
Present study	M	237	170.4±0.84	107.04±2.80	18.81±1.77
	F	156	165.33±0.83	105.99±4.10	20.90±2.91
1976–1979	M	76	169.01±1.70	101.70±2.46	23.40±2.63
	F	127	169.34±1.06	101.50±2.80	21.83±2.40
1976–1978	F	82	171.34±1.22	101.26±3.00	22.25±2.83
1977–1979	F	45	165.78±1.87	100.27±7.02	22.89±5.16

Note: Values are means ± standard errors.

biological meaning. Therefore we reparameterized the curve (Appendix 1), so that t_{zero} and k were functionally replaced by l_0, the estimated absolute length at birth (cm) and k_0 the absolute growth rate at birth (cm·year^{-1}). We expected to be able to fit common values for l_0 and k_0 to the two sexes.

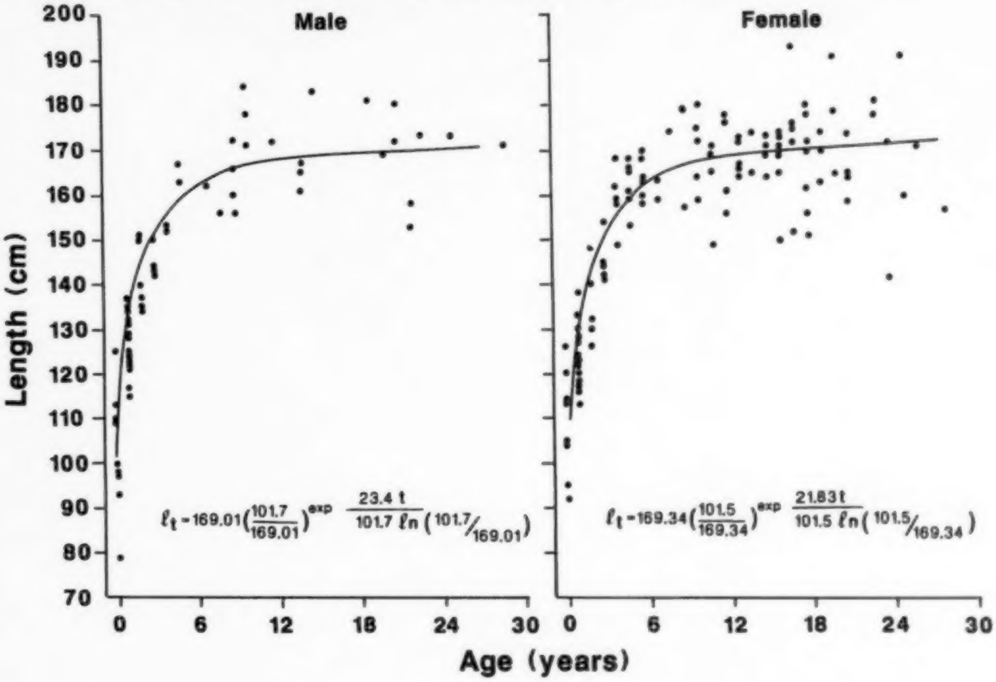
Body weight was related to length using weight = 10^a length^b (Innes et al. 1981). Body weight, length, and axillary girth relationships were also examined by calculating a volume index using weight = length × axillary girth squared (Hofman 1975; Castellini and Calkins 1993).

Growth curves were fitted using the multivariate secant method in procedure NLIN (SAS Institute 1987). Differences between fitted curves were tested by fitting a separate line to each sample, then a single line to the pooled samples and using standard variance-ratio tests (Snedecor's "*F*") on the change in sum of squares about the fitted model (Snedecor and Cochran 1980). Mass-volume relationships were examined by regressing body weight on the volume indices and forcing the regressions through the origin. A Kolmogorov–Smirnov test was used to compare age distributions between sexes (SAS Institute 1987). Condition indices were compared between years using an analysis of covariance (SAS Institute 1987). Day of the year, using 1 January as day 1 was the covariate. Least square means were calculated to isolate differences between group means and elevations. All differences were significant at $p < 0.05$ unless stated otherwise.

Results

The distribution of ages of males and females were examined by year of collection. Significant differences in age

Fig. 2. Length at age for male and female harp seals collected during 1976–1979.



distributions were only observed between males and females collected in 1976 and in 1978. Both years were characterized by large numbers of young males and an absence of old males in the samples (Table 2).

The Gompertz equation fitted the 1988–1992 data well with coefficients of variation (standard deviation of the points about the fitted line divided by the estimated asymptotic length) of 4.9 and 4.6% for males and females, respectively (Fig. 1). Significant differences were observed between growth curves for males and females ($F_{3,390} = 6.58$, $p < 0.05$).There were no significant differences between the sexes for estimated length at birth or growth rate at birth, but males continued growing for a longer period of time resulting in an asymptotic length approximately 5 cm greater than females (Table 3). To determine if the fit of the curves to the data could be improved, we pooled the 1988–1992 data, kept a common birth length and rate of growth for the pooled sample, but recalculated separate asymptotic lengths for each year. This did not result in any significant improvements to the models for either males or for females.

The Gompertz equation also fitted the 1976–1979 data well with coefficients of variation about the fitted lines of 4.6 and 4.9% for males and females, respectively (Fig. 2). However, no significant differences were observed between the male and female growth curves ($p > 0.05$). Both sexes attained an asymptotic length of 169 cm (Fig. 2, Table 3). We also fitted separate asymptotic lengths to each year in the 1976–1979 sample, keeping a common birth length and growth rate for the pooled sample. Significant improvements in the fitted lines were obtained by calculating separate asymptotic lengths for each year for both males ($F_{3,69} = 3.13$) and females ($F_{3,121} = 3.11$).

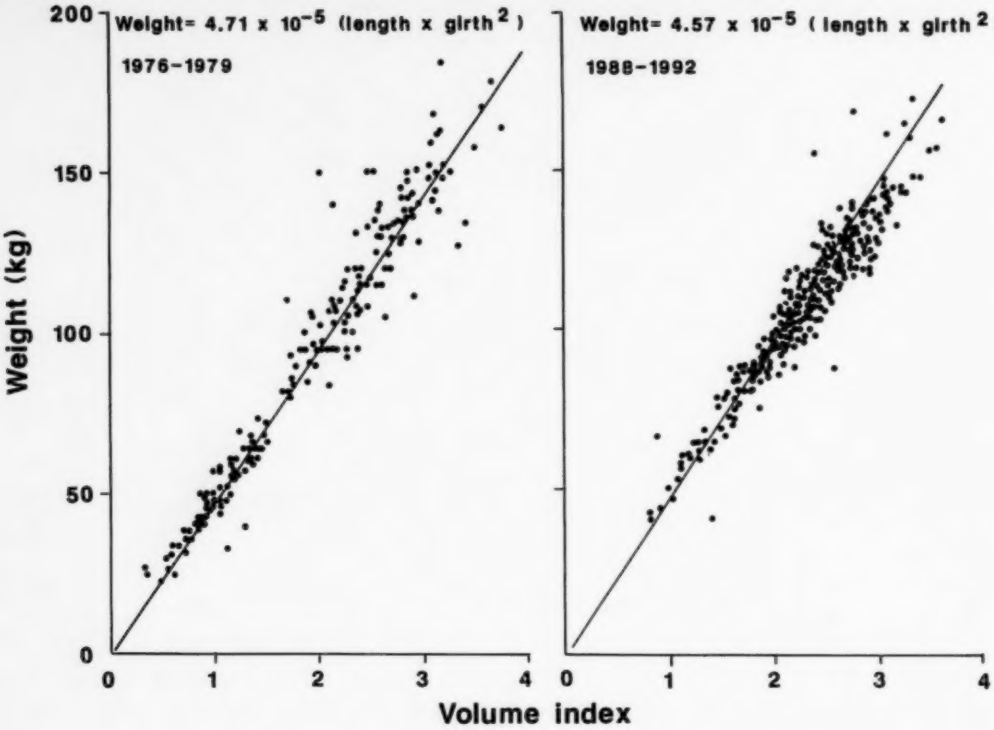
Significant differences were found when growth curves were compared between females collected during 1976–1979 and 1988–1992 ($F_{3,280} = 3.83$, $0.01 < p < 0.05$) owing to the greater asymptotic length obtained in females from the 1976–1979 data set (Table 3). In males, no significant differences were observed between growth curves from the 1976–1979 and 1988–1992 samples.

Samples collected in 1976 and 1978, the 2 years where significant differences were observed in the distribution of ages between males and females were also characterized by having many long females. The years 1977 and 1979 were pooled, as were the samples from 1976 and 1978. Asymptotic lengths of females collected in 1977 and 1979 were similar to asymptotic lengths of females collected during 1988–1992 but were significantly less than the asymptotic lengths for females from 1976 and 1978 ($F_{2,121} = 2.68$, $F_{\text{critical}} = 2.68$, $0.05 = p < 0.1$) (Table 3).

Body weight was related to length by the relationship: weight = $10^{-3.54}$ (length$^{2.52}$) in 1988–1992. For animals collected in 1977–1979 the relationship was weight = $10^{-3.99}$ (length$^{2.73}$). Significant differences were observed in weight–length relationships between the 1976–1979 and 1988–1992 data sets ($F = 4.2$, $F_{2,609} = 3.0$, $p < 0.05$). No differences were observed between the exponent 2.52 and 2.73, but they were significantly different from 3 ($p < 0.05$).

Body weight for the 1976–1979 and 1988–1992 data was related to length and girth by the equation weight = 4.61×10^{-5} (length × girth^2) (SE = 1.5×10^{-7}, $N = 604$, $p < 0.05$). The weight of animals collected during the

Fig. 3. Body weight – volume index relationships for harp seals collected during 1976–1979 and during 1988–1992.



1970s was related to length and girth by the relationship weight = 4.71 × 10^{-5} (length × girth^2), (SE = 3.3 × 10^{-7}, $N = 204$, $p < 0.05$). The weight of animals collected during 1988–1992 showed the relationship weight = 4.57 × 10^{-5} (length × girth^2), (SE = 1.5 × 10^{-7}, $N = 400$, $p < 0.05$). These two regressions were significantly different, suggesting a difference in shape between animals from the two samples and that for any given volume index, animals collected during the 1970s would weigh approximately 5% more than animals collected during 1988–1992 (Fig. 3).

The possibility of changes in weight to volume relationships warranted a more detailed examination of condition. A condition index was obtained using the relationship condition = weight × 10 000/length$^{2.6}$, where the exponent 2.6 was the mean of 2.5 and 2.7, from the weight–length relationships examined above. Condition was examined in animals sampled during March on the whelping patch (Table 4). Among all animals, considerable year-to-year variability in condition was observed. Among males, animals from the 1988–1992 sample were generally in poorer condition than animals from the 1976–1979 sample, but the results are inconclusive owing to small sample sizes for 4 of the 9 years. Among females, samples were available from the whelping patch for all years except 1988, although very few animals were available for 1977. Females collected in 1978 were in significantly poorer condition than females collected in 1976 and 1979, but no significant differences were observed between the remaining years in this sample. In the 1988–1992 sample, females collected in 1992 were in significantly poorer condition than females collected in 1990, but no differences were observed between the remaining years in this sample. Comparing between samples, animals collected during 1988–1992 were in significantly poorer condition in 4/4 years compared to 1976, 2/4 years compared to 1977, 0/4 years compared to 1978, and 3/4 years compared to 1979 (Table 4).

Discussion

Growth curves fitted to length at age data provide a convenient means of summarizing growth using a small number of parameters. However, because they describe growth as changing smoothly with time they cannot be used to track temporary variations in growth rate (Kingsley et al. 1988). We did not examine growth by following individual cohorts through time but instead examined the cross-sectional relationship between length and age in samples collected over periods of 4–5 years. The resulting curves reflect average environmental conditions faced by several cohorts within a population rather than specific conditions encountered by following a single cohort.

Several factors will influence the analysis of growth patterns. These include the age structure of the sample, between-year variability in birth dates, and incorrect estimation of age. In the Northwest Atlantic harp seal, pupping begins in late February in the Gulf, and continues until

Table 4. Year, sample size, and least square mean condition indices (±SE) (analysis of covariance) for male and female harp seals collected on the whelping patch during March.

	Males			Females		
Year	N	Condition index	Significance	N	Condition index	Significance
1976				25	2.23±0.05	1978, 1989, 1990, 1991, 1992
1977	7	2.19±0.07	1989, 1991	3	2.18±0.15	1989, 1992
1978	14	2.09±0.08		34	1.89±0.05	1976, 1979
1979	2	2.10±0.13		25	2.14±0.05	1978, 1989, 1990, 1992
1989	93	1.93±0.02	1977, 1990	60	1.86±0.03	1976, 1977, 1979
1990	18	2.06±0.06	1989	31	1.96±0.05	1976, 1979, 1992
1991	6	1.95±0.08	1977	8	1.94±0.09	1976
1992	18	2.02±0.04		12	1.72±0.07	1976, 1977, 1979, 1990

Note: Years listed in the significance column indicate significant differences at $p < 0.05$.

late March at the Front (Sergeant 1991). We assigned a mean birth date of 2 March (Stewart et al. 1989) calculated for animals from the Gulf. In our sample, differences between actual birth dates and the mean date used here would increase the variance associated with the growth curve parameters but are unlikely to affect the bias of growth estimates (Leberg et al. 1989). More important are the effects of age distribution in the sample and errors in age determination that will tend to lead to biased estimates of growth parameters (Leberg et al. 1989). Errors in age determination owing to difficulties in determining age in animals >4+ years (Bowen et al. 1983) and the lack of many young animals in our collections are responsible for the differences in our estimated length at birth of >100 cm (Table 3), and the observed length of 78 cm at birth or 88 cm at weaning (Kovacs and Lavigne 1985). Errors in age determination will have little effect on estimates of asymptotic lengths that tend to be the most robustly estimated parameter and are little influenced by ageing errors (McLaren 1993). Furthermore, the large number of older animals in our samples will likely improve our estimate of this parameter. During review, one referee suggested that alternative models to the Gompertz curve may have been more appropriate. In response to this suggestion we examined other models from the Richard's curve family and concluded that for the type of age–length data commonly collected for marine mammal studies, the von Bertalanffy or Gompertz curves would both provide reasonable fits to the data (Appendix 1).

We were unable to determine whether animals collected in this study were originally from the Gulf or from the Front, or how much time each had spent in the respective areas. Consequently, we have assumed that there is no difference in growth patterns between the two populations. Although there is considerable overlap in summer distribution between the two groups, some harp seals born in the Gulf winter on the Front as juveniles, while the converse does not appear to occur (Sergeant 1991). Differences in habitat conditions could affect estimates of growth parameters, but we are unable to test this hypothesis with the available data. In the 1976–1979 sample, only 54% of the animals were collected in the Gulf, while in the 1989–1992 sample 97% of the animals were collected in the Gulf.

In an earlier analysis of harp seal growth, no evidence of sexual dimorphism in any growth parameter was observed (Innes et al. 1981). Innes et al. (1981) also concluded that harp seals sampled in the 1970s had a larger body size in age-class zero and reached a greater asymptotic length than did a sample of animals obtained in the 1950s (Innes et al. 1981). Using a larger data set, our analysis indicates that although the differences are slight (3%), adult male harp seals are significantly longer than adult females. The differences in findings between the two studies arise from the presence of very large females in samples collected in 1976 and in 1978. Male and female harp seals collected in 1977 and 1979 and 1988–1992 had similar age distributions. In the 1976 and 1978 samples, significant differences were observed in the distribution of ages between males and females. In these samples the majority of females were collected on the whelping patch as part of early studies on lactation energetics in harp seals, while the majority of males were killed in the Arctic or in the Gulf after whelping had finished. Asymptotic lengths of the 1977–1979 sample were similar to asymptotic lengths for 1988–1992. Our estimated asymptotic length of 165 cm is longer than the 161 cm estimated (Innes et al. 1981) for female harp seals sampled during the 1950s (Laws 1959). However, it is unlikely that this comparison is valid owing to the small sample size ($N = 8$; Innes et al. 1981) and errors in age determination. In this early sample, animals were aged by pelage patterns (Laws 1959; Sergeant 1991), which would be less accurate than age determination using sectioned teeth. We conclude that no change has occurred in asymptotic lengths of male and female harp seals since the end of the commercial hunt in 1985.

Body weight is probably the best measure of size for many purposes (McLaren 1993). Unfortunately, seasonal changes in body weight occur (Beck et al. 1993*a*) in relation to the animal's age, sex, and reproductive condition. Among pinnipeds the pattern of weight loss during the breeding season differs between sexes. During the 12-day lactation period female harp seals lose on average 3.1 $kg \cdot d^{-1}$ (Kovacs et al. 1991). No data are available for male weight loss during the 3-week breeding season. In male grey (*Halichoerus grypus*) (Anderson and Fedak 1985) and elephant seals (*Mirounga angustirostris*) (Deutsch et al. 1990), the rate of weight loss per day is considerably less than that observed in females, although total weight loss by some males during the breeding season is comparable to the total weight loss of females. Therefore examining age to body weight relationships in which the majority of samples are taken from the whelping patch is not valid unless appropriate correction factors can be applied.

In our weight–length comparisons the exponents 2.5 and 2.7 were not significantly different from the exponent 2.52 observed by Ryg et al. (1993) (taken as the inverse of the exponent 0.397 in their equation 7: $L = 0.253\ \text{weight}^{0.397}$) in a multispecies comparison of pinnipeds and cetacea, which did not include harp seals. However, these exponents are significantly different from 3, indicating that growth in mass is not isometric.

Assessing body condition has often been suggested as a means of monitoring animal populations (Hanks 1981; McLaren and Smith 1985). Among pinnipeds, various measures have been proposed incorporating a measure of "fatness" such as girth, blubber thickness, and total body or sculp weight corrected by some measure of body size, usually body length (Innes et al. 1981; Stewart and Lavigne 1984; Ryg et al. 1990; Beck et al. 1993a). Using the index body weight/length$^{2.6}$, our data suggest that there has been a decline in harp seal condition on the whelping patch since the 1970s. However, these findings must be considered as tentative owing to possible sampling biases related to the rate of male and female weight loss, between-year differences in sampling dates or methods used to collect samples. In the 1976–1979 samples, animals collected in March were shot on the ice. In the 1989–1992 March samples, animals were shot and recovered in the water. If thin females are more likely to enter the water to feed, then our sampling would have selected animals in poorer condition. Conversely, our sampling would still indicate a change in condition if lactating females were spending more time in the water than during the 1970s. Stewart and Murie (1986), in a sample of lactating females collected on the ice between 1976 and 1984 and including some of the animals used in our analyses, reported that 23% of the females had food in their stomachs with a mean weight 22 g (SD = 82, $N = 45$). Beck et al. (1993*b*) found that 72% of lactating females shot in the water had food in their stomachs with an average weight of 21 g (SD = 33, $N = 36$). Time-depth recorder records show that females spend approximately 21% of their time hauled out (Lydersen and Kovacs 1993), which is similar to the observed 15% of the time that females spend with their pups from on ice behaviour studies in the late 1970s and early 1980s (Kovacs 1987; Stewart 1987).

This apparent decline in condition coincides with a recent decline observed in the Atlantic commercial fishery. Diet information has shown that harp seals feed predominantly on capelin (*Mallotus villosus*) in the Gulf and off the Labrador coast particularly during their northward migration and very little on Atlantic cod (*Gadus morhua*) (Murie and Lavigne 1991; Beck et al. 1993*b*; Foy et al. 1981). However, recently there are indications that Arctic cod (*Boreogadus saida*) a species with a lower caloric density than capelin (Beck et al. 1993*b*) and normally an important component of the harp seal's Arctic diet (Finley et al. 1990) have become more important in the diet of harp seals collected off southern Labrador (NAFO zones 2J3KL) (Lawson et al. 1993). This would suggest that changes in the availability of capelin to harp seals have occurred, which may have had a negative effect on their condition.

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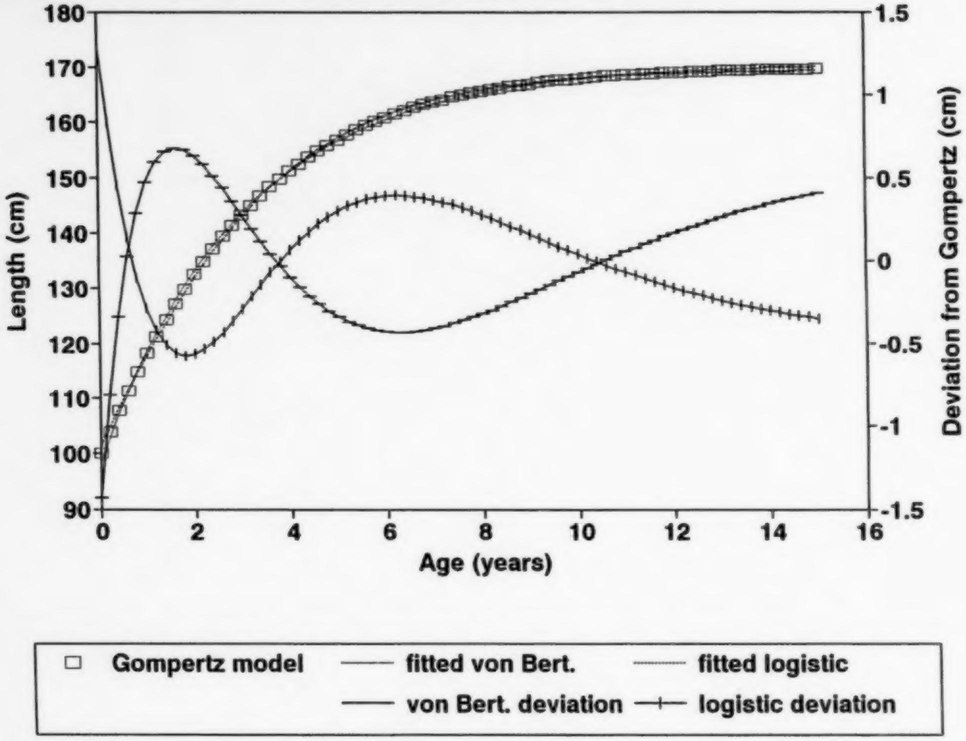
Appendix: Which growth curve best fits harp seal age–length data?

The fitting of asymptotic sigmoidal growth curves should be regarded as a means of summarizing data sets to facilitate their comparison and to provide insights into the functioning of biological systems. As a means of estimating real biological parameters of mammalian growth, growth curve analysis should be used with caution: these are approximate, heuristic models. Therefore, biological conclusions that are highly sensitive to growth model selection should be viewed with caution and carefully checked; conversely, if biological conclusions are robust to the choice of growth model, then it matters little which is chosen.

The curves considered in this Appendix are the von Bertalanffy curve for length, the Gompertz curve, and the logistic curve.

Fig. A1. von Bertalanffy, logistic, and Gompertz growth curves fitted to typical harp seal data and deviations of these curves from values predicted by the Gompertz model.



The growth curve used in this article is the Gompertz curve. This curve is well known in its basic form

$$l_t = L_\infty \exp\left(-a \exp\left(-bt\right)\right)$$

where L_∞ is the asymptote. In this formulation a and b are fitting constants; they are difficult to interpret. The curve can be formulated in terms of any three parameters, within certain constraints, and more useful formulations of the equation are available; for example

$$l_t = L_\infty \exp\left(-\exp\left(-ek(t - t_0)\right)\right)$$

where k is the maximum slope of the curve and t_0 is the abscissa at which this maximum slope is realised. For the present article, the curve has been formulated in terms of the ordinate l_0 and the slope k_0 at zero abscissa (i.e., in biological terms, the absolute length at birth and the absolute rate of growth at birth):

$$l_t = L_\infty \left(\frac{l_0}{L_\infty}\right)^{\exp\left[\frac{k_0 t}{l_0 \ln(l_0 / L_\infty)}\right]}$$

The Gompertz curve is established as a standard curve for describing mammalian (Laird 1966; Heide-Jorgensen 1994; Stewart 1994) and other (Sugden et al. 1981) growth although it has little theoretical justification. It has practical advantages: it is defined, and lies between 0 and L_∞, for all values of the abscissa; fitting algorithms converge reliably without calculation or iteration problems. If growth in length is Gompertz, and growth is isometric and at constant density, then growth in weight is also Gompertz.

Alternative candidate curves are the von Bertalanffy curve for length

$$l_t = L_\infty \left(1 - (1 - (l_0 / L_\infty)) \cdot \exp\left[\frac{-k_0 t}{(L_\infty - l_0)}\right]\right)$$

and the logistic curve

$$l_t = L_\infty / \left(1 + (L_\infty / l_0 - 1) \cdot \exp\left[\frac{-k_0 t}{l_0 (1 - (l_0 / L_\infty))}\right]\right)$$

formulated in terms of the same parameters.

Richards (1959) pointed out that these three are special cases of a general family of sigmoidal asymptotic curves that is defined by variation in the ordinate at the point of maximum slope. For these curves the values are 0 for the von Bertalanffy length curve, which is one extreme of Richards' family; L_∞ / e for the Gompertz curve; and $L_\infty / 2$ for the logistic.

In fitting growth curves to data, it is necessary that the data display the parameters of the curve to be fitted. For example, an asymptotic curve cannot be fitted if there is no asymptote evident in the data (for examples see Stewart 1994 and Heide-Jorgensen 1994). And in the same way, if the data do not show a point of inflection, sigmoidal curves will be difficult to fit, and fitting algorithms may fail to converge. But conversely, data that do display an evident point of inflection *will* readily distinguish between different candidate sigmoidal curves (see, e.g., Sugden et al. 1981). Harp seal age–length data lies entirely on the upper limb of any sigmoidal model and shows no point of inflection; this is true for most marine mammal age–length data. Consequently, such data distinguishes poorly between different members of the Richards family with small values of m such as the von Bertalanffy ($m = 0$), Gompertz ($m = 1$), and logistic ($m = 2$) curves, and it is difficult to fit with the general form of the Richards curve (see, e.g., Innes et al. 1981).

As an example of the interchangeability of these curves for upper-limb data, we constructed a Gompertz model of growth, using parameter values similar to those we fitted to harp seal data ($l_0 = 100$ cm, $k_0 = 20$ cm/year, $L_\infty = 170$ cm; life-span, 15 years), and we then fitted a von Bertalanffy length curve to it. The deviation of the von Bertalanffy curve is shown in Fig. A1; it never exceeds 0.85% of L_∞ and has an RMS of 0.2% of L_∞. Fits of growth curves to mammal length data usually have an RMS deviation of 4.5 to 8% of L_∞. This close fit confirms that if upper-limb data are truly generated by a growth process that follows one of these models, the other will fit them without a noticeable increase in the scatter of points about the line. Which of these curves is "best" is thus apt to be a sterile discussion for data of this kind.

For the data in the present article, we fitted von Bertalanffy, Gompertz, logistic, and extended Richards[2] curves to each sex and sample. The logistic curve gave fits that were slightly worse than Gompertz in all cases (Table A1). The Richards curve was fitted in all cases, but the standard error on the shape parameter m was so large that the fitted Richards curves are only significantly different from either von Bertalanffy ($m = 0$) or Gompertz ($m = 1$) curves in one case: 1976–1979 males. The standard deviation of points about the line is tabulated as one criterion for evaluating the fit of the curves. In three cases, viz, 1976–1979 females and both sexes for 1988–1992, the criterion is practically the same for all the curves considered; only in the case of the 1976–1979 males, which had a number of very short, young animals, is there a consistent cline toward the extended Richards curve. For all samples, l_0 decreases with m, while k_0 increases with decreasing m; the standard errors of l_0 and of k_0 increase with decreasing m. Where the Richards curve fits a large negative m with a large k_0, the standard error of k_0 is also large, to the extent that the confidence regions overlap. The growth pattern parameters estimated using the Gompertz curve have the smallest standard errors, and also the smallest error correlations, except in the case of the 1976–1979 females where the Richards curve fitted lies between Gompertz and logistic ($m = 1.16$).

We conclude that the question of which asymptotic curve best fits harp seal length data is difficult to answer within the limitations of typical field data. There is so little difference between the candidate curves within the range of such data sets that the decisions on whether to fit complex curves with extra parameters will always be temperamental and unpredictable. It is clear, however, that an arbitrary choice of either

Table A1. Parameters for logistic, Gompertz, von Bertalanffy, and extended Richards curves fitted to length-at-age data for northwest Atlantic harp seals measured in 1976–1979 and in 1988–1992.

	Logistic				Gompertz				von Bertalanffy				Extended Richards				
	L_∞ (cm)	l_0 (cm)	k_0 (cm/year)	SD (cm)	L_∞ (cm)	l_0 (cm)	k_0 (cm/year)	SD (cm)	L_∞ (cm)	l_0 (cm)	k_0 (cm/year)	SD (cm)	L_∞ (cm)	l_0 (cm)	k_0 (cm/year)	m	SD (cm)
1988–1992																	
Males	170.16	108.31	16.50	8.33	170.40	107.04	18.81	8.31	170.70	105.66	21.95	8.30	170.92	104.75	24.50	−0.624	8.31
	(0.81)	(2.67)	(1.42)		(0.84)	(2.80)	(1.77)		(0.88)	(3.00)	(2.31)		(1.12)	(3.75)	(7.53)	(1.588)	
Females	165.19	107.36	18.19	7.58	165.33	105.99	20.90	7.55	165.48	104.43	24.66	7.53	166.04	99.51	46.04	−2.714	7.52
	(0.81)	(3.93)	(2.28)		(0.83)	(4.10)	(2.85)		(0.85)	(4.33)	(3.75)		(1.12)	(5.95)	(25.51)	(1.904)	
1976–1979																	
Males	168.72	102.97	20.69	7.85	169.01	101.70	23.40	7.71	169.40	100.31	27.09	7.58	171.48	94.41	55.27	−3.261	7.37
	(1.69)	(2.38)	(2.22)		(1.70)	(2.46)	(2.63)		(1.72)	(2.56)	(3.24)		(2.41)	(4.26)	(23.09)	(1.315)	
Females	169.18	102.91	18.99	8.35	169.34	101.50	21.83	8.35	169.55	99.81	25.86	8.36	169.31	101.79	21.20	1.196	8.38
	(1.04)	(2.61)	(1.92)		(1.06)	(2.80)	(2.40)		(1.05)	(3.10)	(3.25)		(1.13)	(4.16)	(7.00)	(2.113)	

Note: The logistic curve is Richards with $m = 2$; Gompertz is the limit for the Richards curve as m tends to 1; von Bertalanffy for length is Richards with $m = 0$. Extended Richards means that the parameter m that determines the shape is permitted to be negative (Richards' 1959 definition limits m to be non-negative.) Negative m is equivalent to case 2 of Schnute (1981, p. 1133). SD is the standard deviation of points about the fitted curve. This is a good measure of fit for growth curve analysis. Values in parentheses are standard errors. Standard errors increase with decreasing m, but error correlations also increase, so the generalized variance changes little.

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[2] Richards specified his family of curves to be bounded at one limit by the von Bertalanffy curve for length. If this limit is transgressed, the curve has an infinite slope at zero ordinate, but may nevertheless be fitted to data in regions where the ordinate is greater than zero.

Gompertz or von Bertalanffy curves will usually fit as well as a more complex curve such as the Richards curve.

Practical considerations, such as the standard errors and error correlations of the resulting estimates, should play a part in the choice of curve. As pointed out at the beginning of this Appendix, if the biological conclusions reached by a study are robust to the choice of growth model, that choice becomes less critical. However, in the long term, there is value in having published growth analyses use a small range of models so that consistent and mutually convertible results accumulate in the literature. It is also useful if models are formulated in terms of interpretable parameters, with recognizable units.

Upper thermal limits on the oceanic distribution of Pacific salmon (*Oncorhynchus* spp.) in the spring

D.W. Welch, A.I. Chigirinsky, and Y. Ishida

Abstract: Pacific salmon are normally thought to be distributed throughout the Subarctic Pacific, an area where they form the dominant fish fauna. We use a series of generalized additive models to show that salmon exhibit a sharp step-function response to temperature in the oceanic eastern north Pacific in spring. The critical temperature defining the southern boundary varied by species: 10.4°C for pink and chum salmon, 9.4°C for coho salmon, and 8.9°C for sockeye salmon. These thermal limits occur well to the north of the southern boundary of the Transition Zone, at widely separated geographic positions within the Subarctic Domain, and at temperatures much lower than the lethal upper limit for each species. The sharp decline in abundance with temperature, and the remarkably low temperatures at which the response occurs, suggests that thermal barriers form an effective limit to the offshore distribution of salmon in spring, and can limit the distribution of Pacific salmon to a relatively small area of the Subarctic Pacific. The strength of this response is presumably the direct result of strong evolutionary selection. Future temperature changes in the North Pacific could therefore have a direct impact on the production dynamics of Pacific salmon.

Résumé : On pense généralement que les saumons pacifiques sont répartis dans l'ensemble du Pacifique subarctique, zone où ils constituent les espèces dominantes de l'ichtyofaune. Nous nous servons d'une série de modèles additifs généralisés pour montrer que les saumons présentent une forte réponse à l'échelon de température, au printemps, dans la zone océanique du Pacifique nord-est. La température critique définissant la limite sud varie selon les espèces : 10,4°C pour le saumon rose et le kéta, 9,4°C pour le coho et 8,9°C pour le saumon rouge. Ces barrières thermiques se situent bien au nord de la limite sud de la zone de transition, en des points géographiques nettement séparés au sein du domaine subarctique, et à des températures beaucoup plus basses que la limite létale supérieure de chaque espèce. La baisse marquée de l'abondance en fonction de la température, et les températures remarquablement basses auxquelles la réaction se produit, semblent indiquer que les barrières thermiques forment une limite effective à la répartition hauturière des saumons au printemps, et peuvent limiter la distribution des saumons pacifiques à une zone relativement petite du Pacifique subarctique. La force de cette réaction est probablement un résultat direct d'une forte sélection au fil de l'évolution. D'éventuels changements thermiques dans le Pacifique nord pourraient donc avoir un impact direct sur la dynamique de la production des saumons pacifiques.

[Traduit par la Rédaction]

Introduction

Despite some 40 years of research, the reasons why Pacific salmon undertake their vast ocean migrations are still only poorly understood. The reasons are presumably closely related to their evolutionary biology and production dynamics. Some two-thirds or more of the life history of Pacific salmon (*Oncorhynchus*) is normally spent in the pelagic environment of the open Pacific Ocean, a behavioural choice that is under evolutionary control. During this time most somatic growth is completed (Ricker 1962, 1976), and a significant fraction of total cohort mortality also occurs (Parker 1962; Ricker 1962, 1976). Yet, despite the length of this period in the life cycle, relatively little is known about the biological dynamics underlying the pelagic period of the life history.

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Salmon are widely distributed on the high seas. One relatively tractable aspect of the study of the high seas biology of salmon involves the relationship between the distribution of salmon and physical and biological factors. A better understanding of the factors that restrict the pelagic distribution of Pacific salmon could provide insight into the interaction of Pacific salmon with their environment, particularly with regard to how current predictions of climate change could influence the salmon resources of the North Pacific. Because the behaviour of salmon on the high seas is the result of evolutionary selection, oceanographic factors strongly influencing the distribution of Pacific salmon should also influence their production dynamics, because the same selective forces that lead to dominance of genotypes with high fitness also lead to maximization of population growth rates (Caswell 1989).

Most published work on the high seas biology of salmon dates from the late 1950s and early 1960s, a period when the extensive ocean migrations of Pacific salmon were first documented. These studies established the widespread occurrence of salmon in the surface layer of the open north Pacific ocean, particularly the Subarctic Domain. The Subarctic Domain (Ware and McFarlane 1989) is bounded to the south by the Transition Domain, whose southern boundary is defined in turn by the presence of a vertical 34‰ isohaline structure (Dodimead et al. 1963), with cooler less saline water lying to the north. The southern limit to the distribution of Pacific salmon has been variously identified with the vertical 34‰ isohaline structure (Favorite et al. 1976; Quinn 1990; Nagasawa et al. 1994) or the subarctic front between the subarctic and transition domains (Ignell and Murphy 1993).

The fish fauna of the Subarctic Domain is dominated by Pacific salmon, despite their need to return to freshwater to reproduce (Ware and McFarlane 1989). Pacific salmon are therefore the dominant fauna of a cool lens of relatively fresh ocean water that forms the mixed layer above the more saline (>34‰) deep waters of the North Pacific. However, even within the Subarctic Domain salmon are differentially distributed, by species (French et al. 1976; Neave et al. 1976; Major et al. 1978; Takagi et al. 1981; Birman 1985), temperature (Favorite 1969*a*; Favorite et al. 1976; Birman 1985; Blackbourn 1987; Quinn 1990; Erokhin 1991), and possibly salinity (Favorite 1969*b*).

A descriptive understanding of the distribution and general migratory routes of salmon on the high seas is now generally agreed upon. Manzer et al. (1964) reported that there was an apparent difference in temperature preferences between species, with different thermal ranges evident, and preferences seeming to increase over the spring and summer. Manzer et al. suggested that temperature preferences dropped again in September although ambient temperatures remained high. Birman (1985), Erokhin (1991), and Ishida (1962) suggested that the offshore migration of pink salmon (*O. gorbuscha*) was a seasonal migration "following the movement of waters with temperatures of 4–11°C."

However, mechanistic questions concerning *why* salmon are distributed as they are, and the direct *experimental* measurement at sea of the factors influencing the distribution, have rarely been addressed. General statements about the oceanic distribution of Pacific salmon are useful, but they give little insight as to whether and why specific environmental variables are important to salmon, or how significant they really are.

Whatever the proximate response to physical or biological factors, the mechanisms underlying any distributional patterns are likely to have been strongly shaped by evolutionary selection. Establishing why Pacific salmon exhibit specific distributional patterns may help to predict their population response to climatic change, as well as understanding the evolutionary factors that cause Pacific salmon to undertake their remarkable oceanic migrations.

We re-examine the relationship between the physical distribution of salmon and a range of oceanographic factors as a first step towards developing a better understanding of the effects of climate change on Pacific salmon and the potential impact of the increasing abundance of Pacific salmon on their own production (Pearcy 1992). In particular, we examine the question of whether or not salmon are truly distributed throughout the subarctic Pacific. We show that there is evidence of a sharp barrier limiting the distribution of salmon in the North Pacific Ocean in the spring. However, although the results of high seas surveys clearly show evidence of a sharp barrier, satisfactory analysis of the data depends on three factors: (*i*) establishing a satisfactory means of statistically discriminating between a number of possible factors that can simultaneously influence distribution in complicated nonlinear ways; (*ii*) developing a reasonable model of the uncertainty or error inherent in the individual fishing observations made at sea; and (*iii*) providing a parsimonious biological model of the results. We discuss each of these issues in turn.

Equipment, methods, and data sources

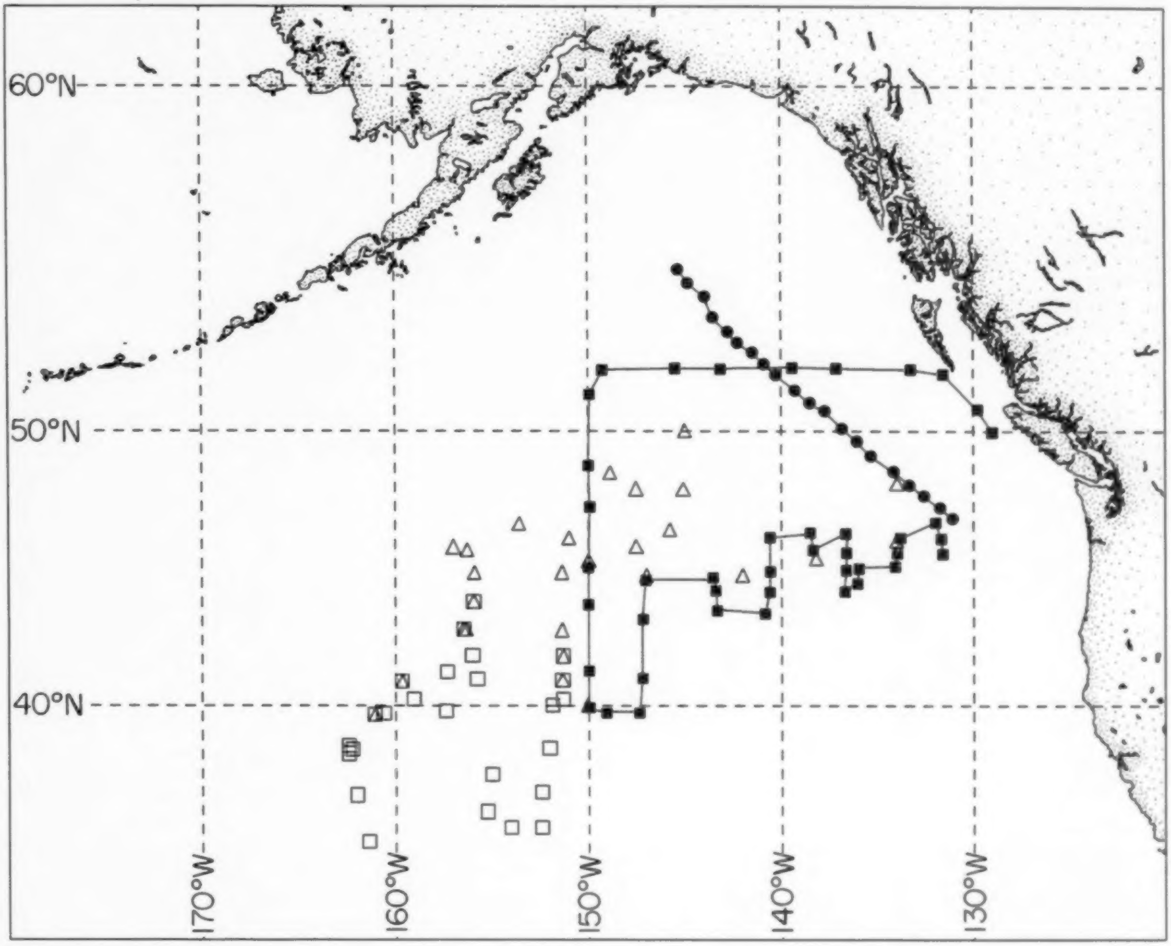
USSR–Canadian Surveys

A joint USSR–Canadian research cruise aboard the USSR research vessel TINRO examined the southern limits to the offshore distribution of Pacific salmon during late spring, 1990. The results reported here are based on data from two cruises, the first occurring from 29 March to 21 April, and involving 20 trawl stations taken along a line between 55°N 145°W and 47°N 131°W (Morris et al. 1992). The second cruise, involving 42 trawl stations, occurred between 27 April and 14 May within a rectangle bounded by the co-ordinates 52°N 129°W and 39°N 150°W (Fig. 1).

Individual north–south transects near the observed southern limit to the salmon distribution were spaced 3° longitude apart and usually involved three stations sampled during daylight hours with a rope trawl. Movement to the next transect was completed overnight. The original cruise plan was intended to extend south of the northern boundary of the Japanese squid driftnet fishing zone in June, at 40°N. However, because of the rapid decline in salmon abundance evident during the first southern transect at 150°W latitude, the southern extent of each planned north–south transect was reduced in order to increase the east–west coverage.

All sampling used a rope trawl, with mouth dimensions 40 m (height) × 60 m (width), and length of 108 m. The net was towed at 5 knots, generally for two contiguous 30-min periods at depths of 20 and 40 m (depth to top of

Fig. 1. Cruise track of the *R/V Tinro* in 1990. ●, location of trawl fishing operations between 29 March and 21 April; ■, locations between 27 April and 14 May. Gillnet (□) and longline (△) samples taken opportunistically by the *W.E. Ricker* in 1987–1990 are also shown.



head rope), for a total sampling time of 1 h. However, in cases where echo sounding indicated that fish were located at a particular depth, trawling was restricted to these strata. This protocol followed standard USSR survey practice. Results presented later are in units of numbers caught per trawl hour.

Vertical profiles to 300 m were made using a Neil Brown CTD probe to record temperature, salinity, O_2, and chlorophyll levels. At all stations the mixed layer depth exceeded 100 m, and the vertical 34‰ isohaline denoting the limit of the Transition Domain lay well beyond the southern-most extent of the survey area (see below).

We used sea surface temperature and salinity as the physical variables to relate to the salmon distribution at each station. Pacific salmon are largely confined to the upper 50 m in the open ocean (Manzer 1964) and all sampling occurred in regions where the mixed layer depth exceeded 100 m, thus justifying the use of single measurements of sea temperature and salinity. Oxygen and chlorophyll measurements showed significant variability with depth and between stations, but a preliminary analysis showed no clear relationship with salmon density, so we did not pursue the analysis of these variables further.

Canadian surveys

During 1987, 1988, and 1990 the Canadian research vessel *R/V W.E. Ricker* collected salmon in southern regions of the NE Pacific using gillnets and surface longlines. Details of these cruises are described elsewhere (LeBrasseur et al. 1987, 1988; McKinnell et al. 1990). Additional data on the distribution of salmon in the NE Pacific conducted between 1961 and 1967 by Canadian and American vessels using surface longline gear is also compiled in Turner and Aro (1968). None of these studies were designed to specifically examine the physical factors limiting the distribution of salmonids on the high seas. However, we use these data to examine the replicability of the results from the *R/V Tinro* cruise.

Statistical methods

Error model

The sampling distribution for Pacific salmon caught on the high seas is heavy-tailed because of the patchy nature of the distribution of salmon at sea. This patchiness directly effects the ability to reliably measure salmon abundance. Ignell and Murphy (1993) found that patchiness (higher variance in estimates of salmon abundance) increased to the south. They concluded that "patchiness directly affects the error structure of any statistical model of salmon abundance near the subarctic frontal zone", and that statistical methods for analyzing the change in salmon abundance must be able to handle this variability—particularly the increase in relative variability that occurs as salmon abundance decreased.

Welch and Ishida (1993) showed that the error structure for high seas salmon catches was closely described by a negative binomial distribution with parameters μ, the true mean abundance or density, and k, the number of degrees of freedom on which the sample is based. This distribution has long tails and at low densities or limited sampling effort is highly asymmetric, probably because of the heterogeneous distribution of salmon into small schools of varying sizes. At large sampling efforts the distribution approaches the Poisson.

Welch and Ishida (1993) found that the spatial coherence of Pacific salmon on the high seas disappeared at separations of ca. 15m. Samples taken at greater spatial separations are therefore statistically independent, and contribute additional degrees of freedom (df) on which observations of salmon abundance or density can be based. This estimate provides a basis for estimating the uncertainty in the trawl survey estimates.

Each one hour tow using the Russian rope trawl results in a single observed catch that is collected over a transect 5 nautical miles (9.26 km) long. The trawl gear therefore provides a single catch equivalent to a set of $k = 9260/15 = 617$ statistically independent samples taken under similar conditions, which provides a sampling distribution very close to the Poisson. For sampling efforts involving large degrees of freedom the negative binomial distribution is very close to the Poisson, but the slightly heavier tails. As a result, the sampling distribution has higher relative variance at the low densities that occur near the limits to the salmon distribution, and therefore captures a number of the important qualitative elements of the sampling data discussed by Ignell and Murphy (1993). We use this estimate of $k = 617$ df in the statistical analysis described later.

GAM analysis

Given an acceptable error model, the question remains of assessing how multiple factors contribute to determining the distribution of Pacific salmon. This issue is particularly important because observations collected at sea cannot be collected according to a preplanned experimental design and are invariably intercorrelated. In addition, some or all of these influences may be nonlinear, and it is important that the nature of this relationship not be distorted by a poor initial choice of a functional model to relate to the observations.

We therefore conducted a preliminary analysis of the relationship between the distribution of Pacific salmon and physical factors using a series of generalized additive models (GAM) (Hastie and Tibshirani 1990; Hastie 1992; Swartzman et al. 1992, StatSci 1993). We define the distribution of salmon in terms of their abundance, expressed as the number of individuals caught at the ith station per trawl-hour, n_i. In general, a GAM model is defined as

$$[1] \quad n_i = \alpha + f_T(T_i) + f_S(S_i) + \cdots + \epsilon_i$$

where $f_T(T_i)$ and $f_S(S_i)$ represent arbitrary smooth univariate functions of temperature and salinity, α represents the mean response (i.e., average abundance), and ϵ_i represents the error distribution for the ith observation. In GAMs, locally weighted fitting is used to calculate a nonparametric estimate of the functional relationship between salmon abundance and potentially significant explanatory variables. The advantage of this approach is that it does not require the prior specification of a series of satisfactory mathematical relationships and instead allows the data to more clearly indicate the important variables and their functional responses.

As negative binomial distributions are not available in current GAM software, we used the Poisson distribution as our error model instead; this assumption is slightly conservative, and results in underestimating the true width of confidence limits on potentially important factors (i.e., overestimating the true number of significant factors; McCullagh and Nelder 1989). However, we use the generalized additive model framework chiefly as a means of identifying candidate factors influencing the salmon distribution and for determining the nature of their influence. The parametric analysis discussed next uses the negative binomial distribution to more accurately calculate the uncertainty in the parameter estimates.

For ease of interpretation, we expressed our abundance observations as $\log_{10}(n_i + 1)$, and used b-splines to form our nonparametric smoothing functions to describe the local influence of salinity and temperature on salmon abundance (Hastie and Tibshirani 1990; Hastie 1992). This model was fit to the $\log_{10}(n_i + 1)$ observations using the statistical modelling language S-Plus (StatSci 1993).

An edge model for the salmon distribution

The GAM analysis presented later indicates that salmon density is related to ambient temperature but not to other measured factors. This relationship appears as a threshold, with no discernable influence below a specific value and a strong negative influence above. To better define the influence of temperature on the southern limit to the distribution of salmon, and to provide a biological interpretation of our observations, we now describe a parametric mathematical model. We concentrate on defining the southern boundary, where the effect of temperature is most pronounced.

Regression analysis when Poisson or negative binomially distributed errors occur has been discussed by Lawless (1987). We limit our presentation to the derivation of the likelihood equations necessary for our parameter estimation.

A simple functional form relating the observed salmon abundance at the i-th station to temperature, $n(T_i)$, is

$$[2] \quad n(T_i) = \bar{\mu}(1 - \Phi(T_i|\bar{T}, \sigma_T))$$

where $\Phi(T_i|\bar{T}, \sigma_T)$ is the value of the cumulative normal probability distribution with parameters $\bar{T}$ and σ_T at temperature T_i, and $\bar{\mu}$ is the mean salmon density in the interior of the geographic distribution.

Equation 2 posits that there is some mean threshold temperature level $\bar{T}$ which salmon actively avoid exceeding, and some normally distributed variance in the response of individual salmon to this temperature threshold, σ_T. The average population response is then obtained by integrating over the behaviour of all individuals, which results in the cumulative distribution (Fig. 2). The mean temperature therefore defines the threshold value at which the *location* of the edge of the salmon distribution is fixed, while the variance will influence the *rate* at which abundance declines near the edge of the distribution. For example, if $\sigma_T = 0$, and all individuals respond exactly alike, then the edge will be a step function located at $\bar{T}$, with abundance dropping to zero instantaneously. In general, 95% of the decline in abundance will occur over an interval of $\pm 2\sigma_T$ centered on this mean, or roughly a two order-of-magnitude decline in abundance.

The third parameter, $\bar{\mu}$, describes the mean abundance in interior regions, away from the edge of the salmon distribution. This value is affected by both gear efficiency and year-to-year fluctuations in abundance. As we are primarily interested in describing the limits to the salmon distribution, and the GAM analysis indicates that the effect of temperature is most reasonably modelled as a threshold, we treat the mean abundance in the interior as a nuisance parameter, and concentrate on the estimation of the parameters $\bar{T}$ and σ_T.

Equation 2 is a biologically based parametric model of the salmon distribution. However, it still lacks a firm statistical basis for estimation. As discussed above, the uncertainty in the salmon catches can be described by the negative binomial distribution. We therefore calculated final parameter estimates and associated confidence intervals for the effect of temperature initially identified in the GAM analysis using maximum likelihood estimation.

Assuming that the negative binomial distribution holds, the exact likelihood equation is derived as follows. The probability of observing n_i fish at the *i*th station when the true abundance is μ_i and the sampling effort is $k_i = 617$ df is

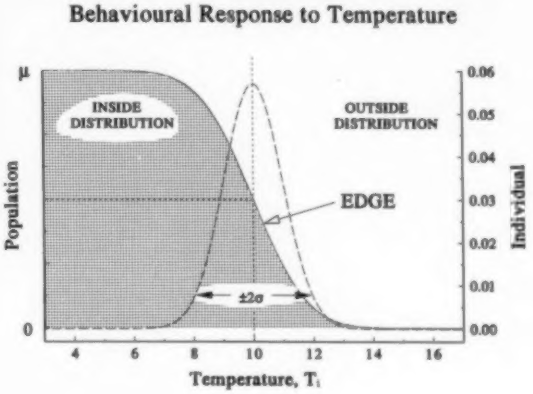
$$[3] \quad P(n_i|\mu_i, k_i) = \frac{\Gamma(n_i + k_i)}{\Gamma(n_i + 1)\,\Gamma(k_i)} \left(\frac{k_i}{\mu_i + k_i}\right)^{k_i} \left(\frac{\mu_i}{\mu_i + k_i}\right)^{n_i}$$

where the Γ function is the analytic continuation of the factorial function for non-integer real numbers and, for integer x, $\Gamma(x + 1) = x!$.

The likelihood of observing a set of n_i catches at the $i = 1, \ldots, m$ stations when the true abundances are μ_i is therefore

$$[4] \quad \mathscr{L}(n_i|\mu_i, k_i) = \prod_{i=1}^{m} \frac{\Gamma(n_i + k_i)}{\Gamma(n_i + 1)\Gamma(k_i)} \left(\frac{k_i}{\mu_i + k_i}\right)^{k_i} \left(\frac{\mu_i}{\mu_i + k_i}\right)^{n_i}$$

Fig. 2. A behavioural model of the edge of the salmon distribution. Individual salmon actively avoid temperatures greater than some threshold; individual variation in the temperature at which this response is expressed results in a range of temperatures over which abundance changes. As temperature increases, a greater fraction of the total population will avoid any given temperature level, leading to a decrease in population density. The rapidity of the decline depends on the degree of individual variation.



The likelihood is therefore dependent on the observations n_i, the sampling efforts or df k_i, and the temperature controlling the true abundance at the *i*th location, $\mu_i(T)$. As noted earlier, the negative binomial distribution reduces to the Poisson distribution in the limit as the df k_i increase infinitely. Thus even gross overestimation of the degrees of freedom is equivalent to assuming that the Poisson distribution applies.

Maximum likelihood estimates of the parameters ($\bar{\mu}$, $\bar{T}$, σ_T) can be obtained by minimizing the negative log-likelihood of the data with respect to the parameters. Substituting the functional form for the parametric temperature–density relationship (2) into the theoretical likelihood (4) and taking the negative of the logarithm,

$$[5] \quad -\ln(\mathscr{L}(n_i|n(T_i), k_i = 617 \text{ df})) = \sum_{i=1}^{m} (n_i + k_i)\ ln(n(T_i) + k_i) - n_i\ ln(n(T_i))$$

where we neglect the contribution of an additive constant and n_i and $n(T_i)$ are the observed and predicted salmon abundances. We used the simplex algorithm (Press et al. 1986) as implemented by Mittertreiner and Schnute (1985), to estimate the maximum likelihood values of the parameters $\bar{T}$, σ_T using [5] and their associated uncertainty.

A critical upper or lower level for some physical factor can be defined as that value at which the decline in density is most rapid. These values correspond to the mean responses $\bar{T}$ within our edge model. We calculate 95% confidence intervals on individual estimates of $\bar{T}$ and σ_T by finding the limits such that twice the difference in log-likelihoods from the maximum likelihood estimates (allowing all other parameters to vary freely) is less than or equal to a $\chi_1^2 = 3.81$ distribution. This calculation is asymptotically

Fig. 3. Vertical sections of salinity and temperature (°C) along 150°W in May 1990. A progressive steepening of the isohalines is evident to the south. *R/V Tinro* stations approached but did not reach the vertical 34‰ isohaline marking the southern boundary of the Transition Zone.

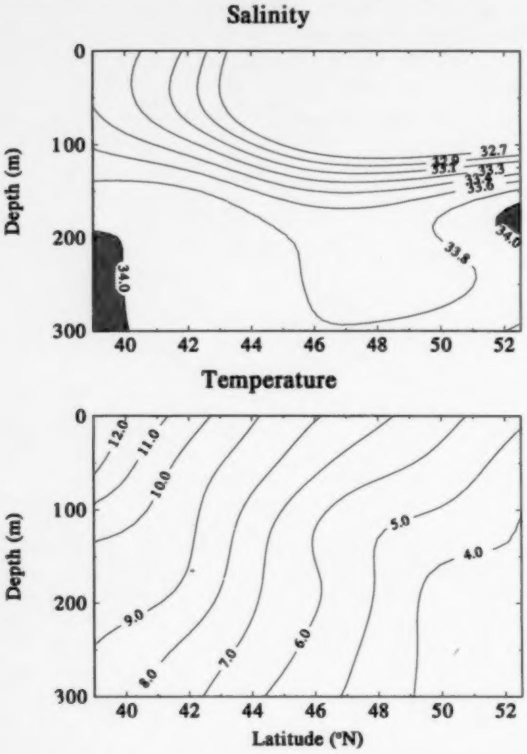
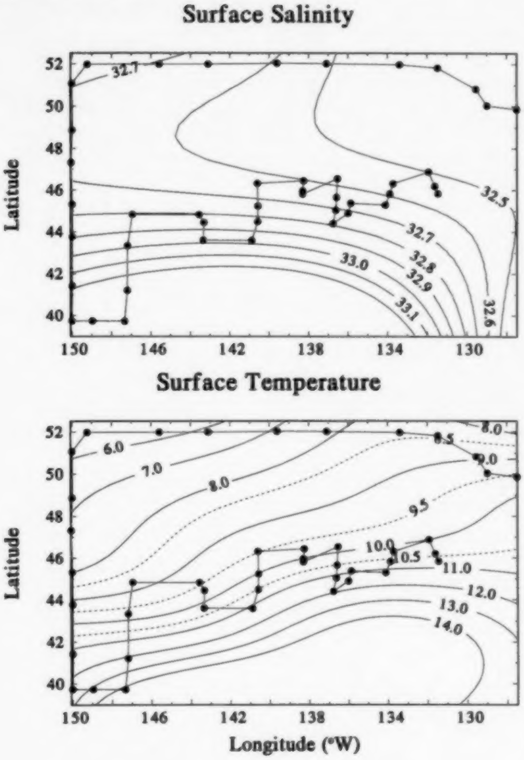


Fig. 4. Contour plots of surface temperature and salinity, May 1990. The second leg of the *R/V Tinro* cruise track is superimposed. Dashed lines on the temperature plot show the approximate geographic location of the thermal thresholds estimated for each species and are discussed later in the text.



correct for large sample sizes (Kendall and Stuart 1979), and is satisfactory for all but very small sample sizes (Lawless 1987). Alternatively, these limits can be thought of in terms of relative plausibility (Edwards 1972). A difference of 3.84 log-likelihood units is equivalent to a difference in relative likelihood of $e^{-3.84} = 0.02$, so parameter values outside the stated range may be thought of as being less than 1/50th as likely to describe the true value of a factor determining the limits of the distribution.

Results

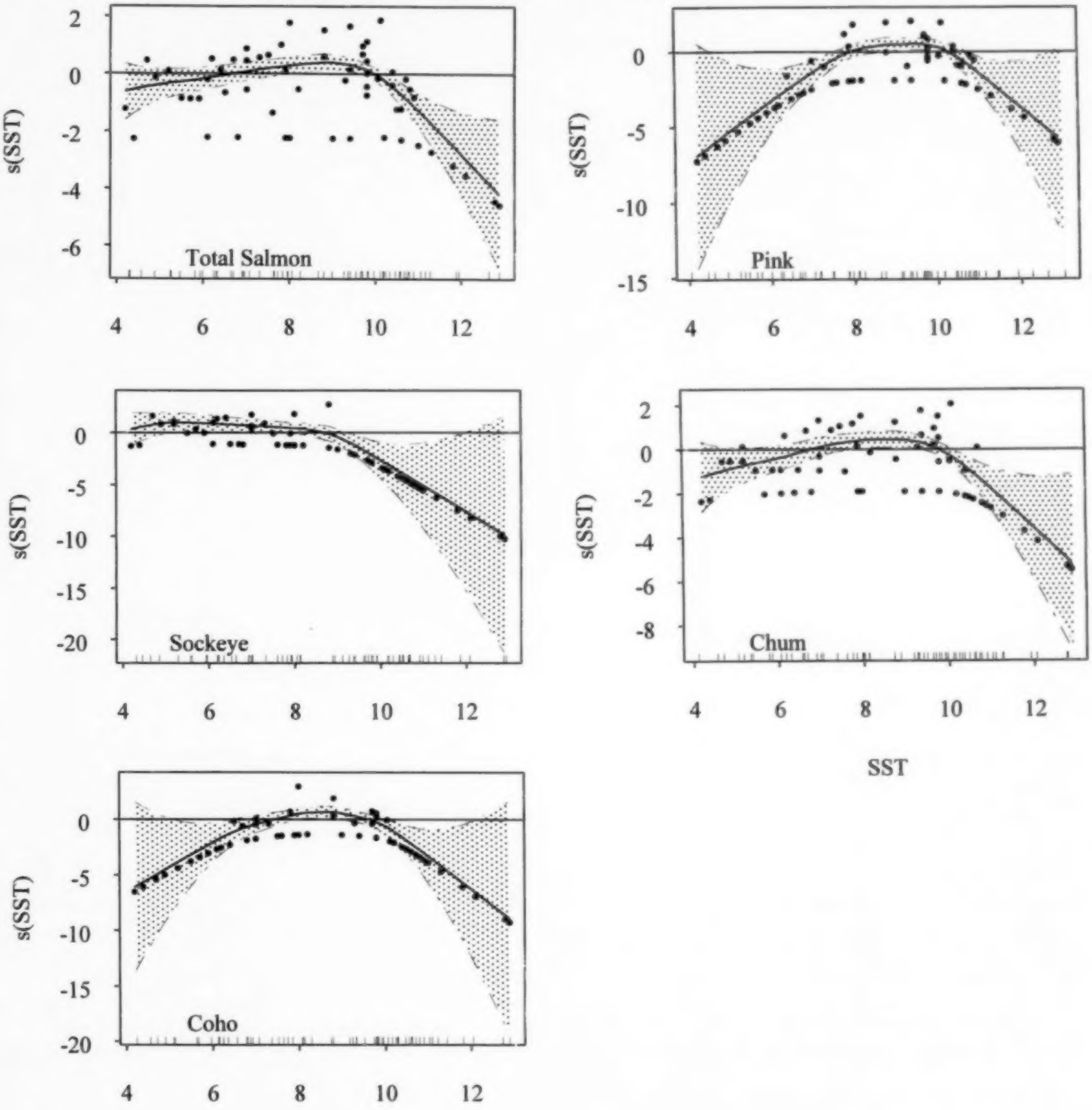
USSR–Canadian surveys

A total of 1912 salmon were caught at 62 trawl stations. Chum (*Oncorhynchus keta*, 749) and pink (*O. gorbuscha*, 725) salmon predominated, particularly in southern regions, but smaller numbers of sockeye (*O. nerka*, 150), coho (*O. kisutch*, 283), and chinook (*O. tshawytscha*, 5) were also taken. The data are reported in Welch et al. (1990).

A vertical section through 150°W and surface maps of temperature and salinity show the major oceanographic features of the region. To the south, isohalines become progressively steeper as the vertical 34‰ boundary defining the southern limit to the Transition Zone is approached, but not reached (Fig. 3). The northern boundary of the Transition Zone (and the southern boundary of the Subarctic Domain) at 150°W is normally defined as occurring at the point where 33.2‰ salinity waters reach the surface (Dodimead 1963), or at about 42°N during our survey. This oceanographic boundary is also normally considered the limit to the distribution of Pacific salmon (Favorite et al. 1976; Blackbourn 1987; Quinn 1990) but, as results reported below show, the observed southern limit to the salmon distribution was species specific, occurring at different geographic positions for different species, and apparently determined only by temperature. Contour maps of surface temperature and salinity (Fig. 4) show that temperature and salinity isoclines were oriented approximately orthogonally, with temperature increasing to the NE as the coast is approached.

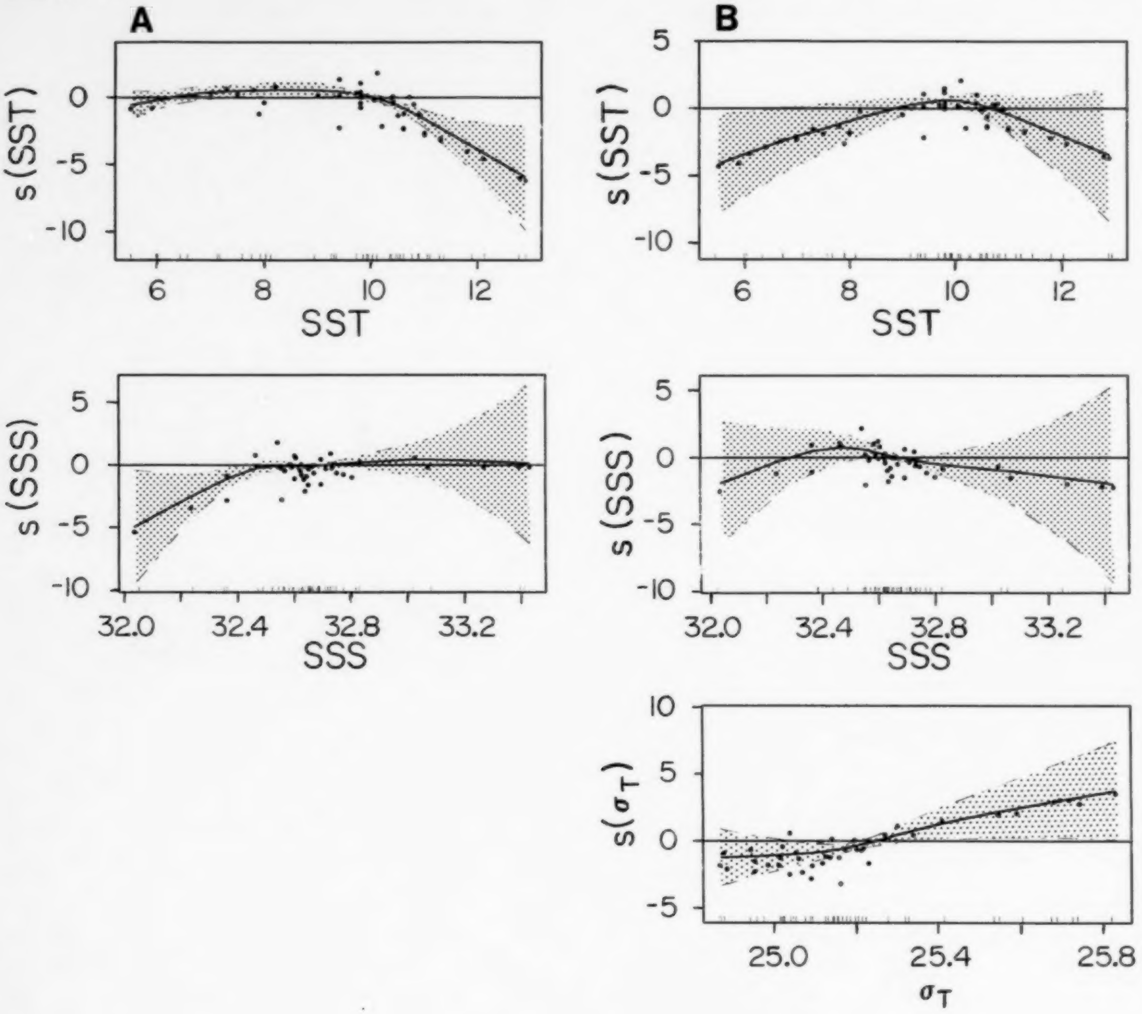
The results of our GAM analyses on the logarithm of salmon abundance are shown in Fig. 5. At ocean temperatures exceeding ca. 10°C a significant negative effect of temperature on total salmon abundance is evident; however, at lower temperatures the estimated value of the nonparametric function fluctuates around zero and the two standard error bands always include zero. Temperature therefore

Fig. 5. Generalized additive model fits for total salmon abundance and individual species; the point at which temperature has a measurable influence on abundance varies by species. The estimated effect of temperature on log salmon abundance is shown as a solid line along with the twice standard error bands about the estimates (shaded area) and the partial residuals (●). The solid line does not fluctuate significantly from zero (no effect) until a threshold temperature is exceeded and the estimated effect on salmon abundance becomes sharply negative. Because there is no detectable effect of temperature on salmon abundance at lower temperatures, the behavioural response to temperature is most simply described as a step function, with salmon actively avoiding warmer temperatures once a threshold has been exceeded.



has a negligible affect on the distribution of salmon until a threshold is passed, at which point a pronounced negative influence on abundance is indicated. A similar result is evident when the catch by species is analyzed. Here the temperature at which the threshold is expressed clearly differs by species. Some evidence for a lower thermal limit is also evident for pink and coho salmon, but the measured effect is probably only statistically significant for pink salmon, as the use of the Poisson model in the GAM analysis underestimates the width of the confidence band.

Fig. 6. GAM fits of total salmon abundance as functions of (A) temperature and salinity, and (B) temperature, salinity, and density (σ_T).



Repeating the analysis to simultaneously consider both temperature and salinity gives similar results (Fig. 6A). The twice standard error curves about the calculated influence of salinity on abundance almost always include zero, and over most of the range of observed salinities the calculated influence is in fact zero. (Two near-coastal stations taken in low salinity waters suggest a weak effect of salinity, but the number of observations is too low to allow discriminating between a direct effect of salinity, and an indirect effect caused by proximity to the coast or some other geographic variable). In contrast, the strong negative influence of temperature on abundance shows little change when salinity is considered. An analysis of variance on these results, including a third analysis using surface density (σ_t) as a proxy for water mass origin (Fig. 6B), confirms these observations, and shows that only temperature has a statistically significant influence on salmon abundance (Table 1).

These results indicate that salmon exhibit a species-specific behavioural response to a threshold temperature, and that they actively avoid entering warmer waters. A parametric analysis, assuming a negative binomial error structure and using maximum likelihood estimation, allows us to estimate more precisely the response to temperature. By calculating the likelihood profile with respect to the model parameters we can also obtain an evaluation of the parameter uncertainty, by allowing all other parameters to vary while we examine the effects of temperature and individual variability on the likelihood.

The results show that the influence of temperature is statistically well-defined, with uncertainty in the estimates of the threshold temperature generally less than ±0.1°C

Table 1. Analysis of variance for the generalized additive model analysis.

	df	χ^2	Pr (χ^2)
Intercept	1		
s(T)	3	25.1	0.00001**
s(S)	2.9	5.6	0.12
s(σ_t)	2.9	4.9	0.17

Note: The three nonparametric models listed in the table were fit sequentially assuming a Poisson error model.

(Table 2). Based on the estimates of σ_T, our measure of the variation in the behavioural response to temperature, total salmon abundance falls by 95% (or nearly two orders of magnitude) in $\pm 2\sigma_T$, corresponding to a temperature interval of 0.68°C. At 150°W longitude, this sudden decline in abundance occurs in roughly 60 nautical miles (Fig. 4).

Plots of salmon abundance by species demonstrate the same statistically well-defined relationship between distribution and temperature, but with the maximum likelihood estimates of the upper thermal limit varying between species (Table 2; Fig. 7). For the *R/V Tinro* data, the upper thermal limit are at ca. 10.4°C for pink and chum salmon, at 9.4°C for coho, and at ca. 9°C for sockeye (note, however, that the latter estimate is dependent on a single large catch of sockeye salmon at 8.9°C).

Coho and pink salmon also show evidence of a lower thermal limit of 7–8°C (Fig.7), an effect only strongly supported by the GAM analysis for pink salmon. We calculated the significance of the lower thermal limit by replacing our single-edged model of the salmon distribution by the two-edged model

$$[6] \quad n(T_i) = \bar{\mu}\Phi\left(\frac{T_i - \bar{T}_{Lo}}{\sigma_{Lo}}\right)\left[1 - \Phi\left(\frac{T_i - \bar{T}_{Hi}}{\sigma_{Hi}}\right)\right]$$

Interpretation of the parameters remains largely unchanged, but in this case we substitute [8] instead of [2] in the likelihood equation, and estimate both low and high critical temperatures. The abundance parameter $\bar{\mu}$ now reflects the mean abundance between the edges of the distribution.

Only the pink salmon abundance data could be satisfactorily fit by this model (Table 3).

Canadian surveys

During annual research cruises on the *R/V W.E. Ricker* in 1987, 1988, and 1990, salmon were caught during surface driftnet and longlining operations in southern regions of the subarctic North Pacific (LeBrasseur et al. 1987, 1988; McKinnell et al. 1990). These sampling locations were located west of where the *R/V Tinro* operated and were occupied in June–July rather than April–May. However, if the relationship between temperature and salmon distribution is not an artifact, then it should hold for different gears, and at different times or areas.

An examination of the relationship between abundance (all species) per unit effort (tans (≈50-m panels) of gillnet, and hachi (120 m, 49 hooks) of longlines) and temperature again shows a significant relationship (Fig. 8). Calculating the critical temperature shows a somewhat lower upper thermal limit for these two gear types than that observed using trawl gear (Table 2A). A single anomalous data point is evident, consisting of 10 pink salmon caught with 10 hachi of longline, at 48°N, 134°W. This was the final set, taken on the return leg of the 1990 cruise, closest to the coastal zone (Fig. 1).

Table 2. Maximum likelihood parameter estimates for the threshold temperature.

	$\bar{\mu}$	$\bar{T}$ (°C)	σ_T (°C)
Total salmon catch			
Rope trawl	42.0	10.34	0.17
	(40.0, 44.0)	(10.3, 10.4)	(0.15, 0.19)
Gillnet	192.7	9.4	0.92
	(175.0, 213.9)	(–,–)[a]	(0.83, 1.01)
Longline	105.9	8.2	2.20
	(95.9, 117.3)	(7.4, 9.0)	(1.99, 2.43)
Species			
Chum	16.2	10.4	0.18
	(15.1, 17.5)	(10.4, 10.4)	(0.16, 0.20)
Pink	15.8	10.4	0.16
	(14.7, 17.0)	(10.4, 10.4)	(0.14, 0.18)
Coho	8.4	9.4	0.46
	(7.5, 9.2)	(9.2, 9.5)	(0.41, 0.50)
Sockeye	5.0	8.9	0.01
	(4.2, 5.8)	(8.8, 9.0)	(0.0, 0.1)

Note: Parenthetical values are approximate 95% confidence intervals on the parameter estimates (see text). Rope trawl results are for the 1990 *R/V Tinro* cruise; gillnet and longline results are for the *R/V W.E. Ricker* cruises. Species-specific estimates are based on the *Tinro* data. Degrees of freedom for the three sampling gears was calculated as $k = 617$ df/trawl hour, 3.3 df/tan of gillnet, and 8 df/hachi of longline.

[a] Confidence interval could not be calculated.

1961–1967 high seas data

One further source of data on the relationship between salmon abundance and surface temperature lies in the extensive exploratory salmon surveys done by Canada and the United States between 1961–1967 using surface longline gear (Turner and Aro 1968). Apparently only sea surface temperature data were collected during this period, so a comparison of salmon catches with other physical variables is not possible.

In contrast to the results obtained using data for the 1987–1990 period, analysis of all available data shows no relationship between temperature and salmon abundance (Fig. 9). However, most data were collected during summer months, and included samples taken in coastal regions. Maturing salmon must travel through coastal regions with substantially higher temperatures than the critical values estimated from the *R/V Tinro* data to return to their spawning grounds in the summer and fall.

A plot of all stations sampled in the winter and spring in the offshore region (defined for current purposes as

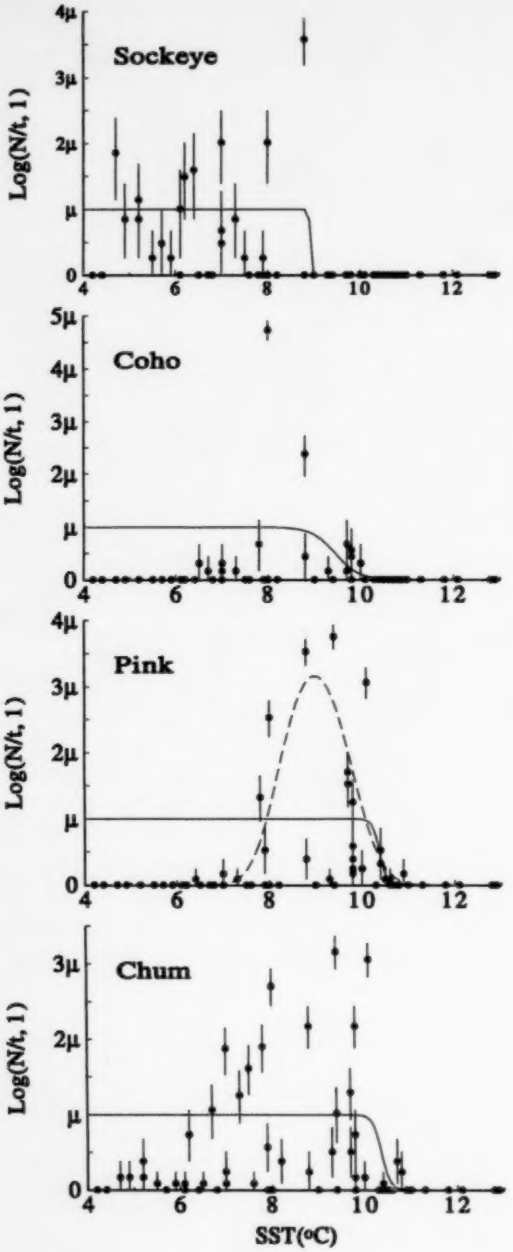


Fig. 7. Salmonid catch versus temperature. Non-zero catches are expressed in terms of the logarithm of numbers trawled, after normalizing by the fishing time (t). Zero catches are plotted as log(1). Under this transformation the vertical range of positive catches is compressed to emphasize the sudden drop in density at the threshold temperature. Vertical bars indicate the 95% confidence limits on the individual catch estimates, calculated using the negative binomial distribution with $k = 617$ df and assuming equal probability tails. Lines show the predicted salmon abundance as a function of temperature based on the maximum likelihood analysis. The dashed line shows the fit of the two-edged thermal limit model described in the text for pink salmon.

those stations west of 135°W and south of 52°N) shows that no fishing occurred in regions warmer than 11°C; thus, no data are available for those areas and times comparable to the more recent studies. This omission is perhaps not surprising, because the 1960s work was primarily concerned with determining where salmon went on the high seas, and not with establishing where the distribution stopped.

Discussion

Our results demonstrate that the high seas distribution of salmon can show extremely sharp species-specific thermal limits in the spring and early summer, a result not previously noted (cf. Burgner 1991). Although salmon are occasionally caught in warmer waters, especially in the coastal zone, the abundance of Pacific salmon appears to drop by one to two orders of magnitude at 10.4°C, with somewhat lower species-specific thermal limits (sockeye, 8.9°C; coho, 9.4°C; pink and chum, 10.4°C). At least in spring and early summer, this thermal barrier confines Pacific salmon to a region smaller than the total area of the Subarctic Domain, the extent of which depends on species (Fig. 10). Pacific salmon thus do not always occupy the entire subarctic Pacific, as they have traditionally been assumed to (Favorite et al. 1976; Ware and McFarlane 1989; Quinn 1990).

However, examination of distributional data collected during the late 1950s and early 1960s (Turner and Aro 1968), demonstrates that the specific thermal limits we find in the offshore either do not hold in coastal regions, or at times other than the spring, early summer, and (possibly) winter. Asian studies of high seas salmon distributions in spring (Mishima 1974, Erokhin 1991) report upper thermal limits similar to those that we find, but do not demonstrate that these limits are either sharp, or that the limit is expressed as a threshold.

The sharpness of the limits, and their expression as a threshold, are clearly demonstrated through our use of generalized additive modelling techniques, and our earlier findings concerning the nature of the uncertainty in estimates of salmon catch on the high seas (Welch and Ishida 1993). GAMs have been used before in spatial analysis of groundfish data (Swartzman et al. 1992). The distinguishing characteristics of our application are (i) the very clear demonstration that the response of salmon to temperature

Table 3. Maximum likelihood parameter estimates for the two-edged distributional model applied to the pink salmon data.

$\bar{\mu}$	$\bar{T}_{Lo}$ (°C)	$\sigma_{T,Lo}$ (°C)	$\bar{T}_{Hi}$ (°C)	$\sigma_{T,Hi}$ (°C)
42.1	8.1	0.49	10.1	0.28
(32.1, 73.8)	(7.9, 8.5)	(0.38, 0.66)	(9.7, 10.2)	(0.18, 0.49)

Note: Parenthetical values show approximate 95% confidence intervals on the parameter estimates (see text).

is expressed as a threshold, with no measurable influence below a specific temperature, and a very sharp population response at the threshold, and (*ii*) the demonstration that other factors measured in our study play little if any role in determining the southern limit to the distribution of salmon in the spring. Making these conclusions would be much more difficult without the use of GAMs, with their ability to incorporate realistic assumptions concerning the nature of the observational errors, and their lack of a priori assumptions about the nature of the functional response between variables. The comparison of cumulative distribution functions of measures of habitat and abundance (D'Amours 1993; Perry and Smith 1994) provide an alternative approach to GAMs but lack an obvious way to incorporate the uncertainty of the catch observations into the analysis.

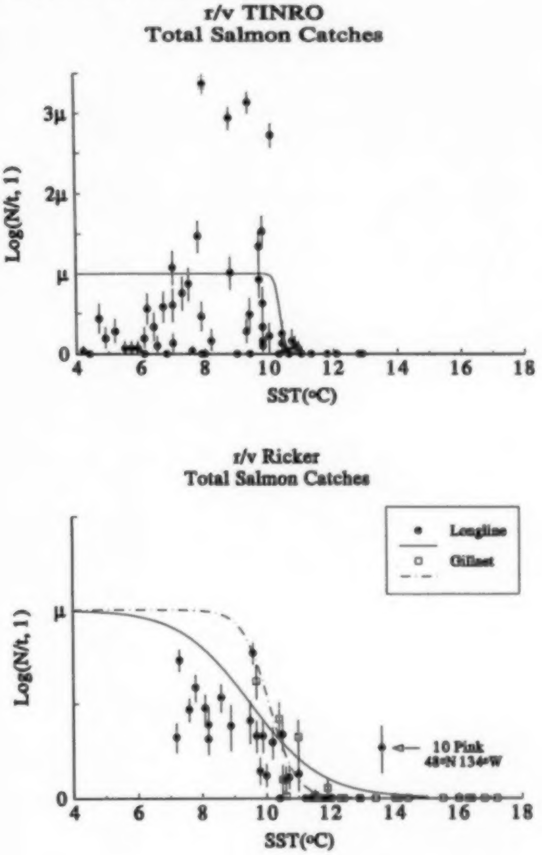
Our current understanding of the factors controlling salmon distributions on the high seas is still too limited to provide a clear understanding of why salmon apparently choose not to occupy the entire subarctic North Pacific, or why their distribution should change with season or region. However, some speculation is possible.

Brett et al. (1969), in a classic study of thermal physiology, demonstrated that the temperature for optimal growth decreased in juvenile sockeye as ration size decreased. Sockeye fed to satiation had a growth optimum at their final thermal preferendum (15°C); however, ration sizes of 1.5, 3.0, 4.5, and 6.0% body weight·day^{-1} had respective temperature optima for growth of 5, 8, 10, and 12°C (Brett et al. 1969). An analogous relationship between increasing ration size and preferred temperature was also found experimentally for lake charr, *Salvelinus namaycush* (Mac 1985).

Brett (1971) concluded that restricted ration dictated much of the energetic physiology of young sockeye in nature. As a result, they were rarely found at the 15°C thermal optimum found in the laboratory. As fish exposed to thermal gradients under laboratory conditions demonstrate distinct thermal preferences, and the ability to discriminate temperature differences as small as 0.03°C (Beitinger and Fitzpatrick 1979), immature salmon maximizing their growth rate under food limiting conditions may therefore have both the ability and the evolutionary selective pressure needed to ensure that they can respond to the fine temperature gradients found on the high seas. Experimental data on behavioural thermoregulation by fish generally indicate that the preferred temperature selected by fish is similar to the temperature yielding optimal growth rates (McCauley and Casselman 1981; Jobling 1981).

Temperature optima for juvenile sockeye fed rations of 1.5–3% of body weight·day^{-1} are similar to the temperatures at which adult sockeye were distributed in the spring in this study. A testable hypothesis is therefore that upper thermal limits for salmon in the North Pacific should vary with food intake, and therefore season.

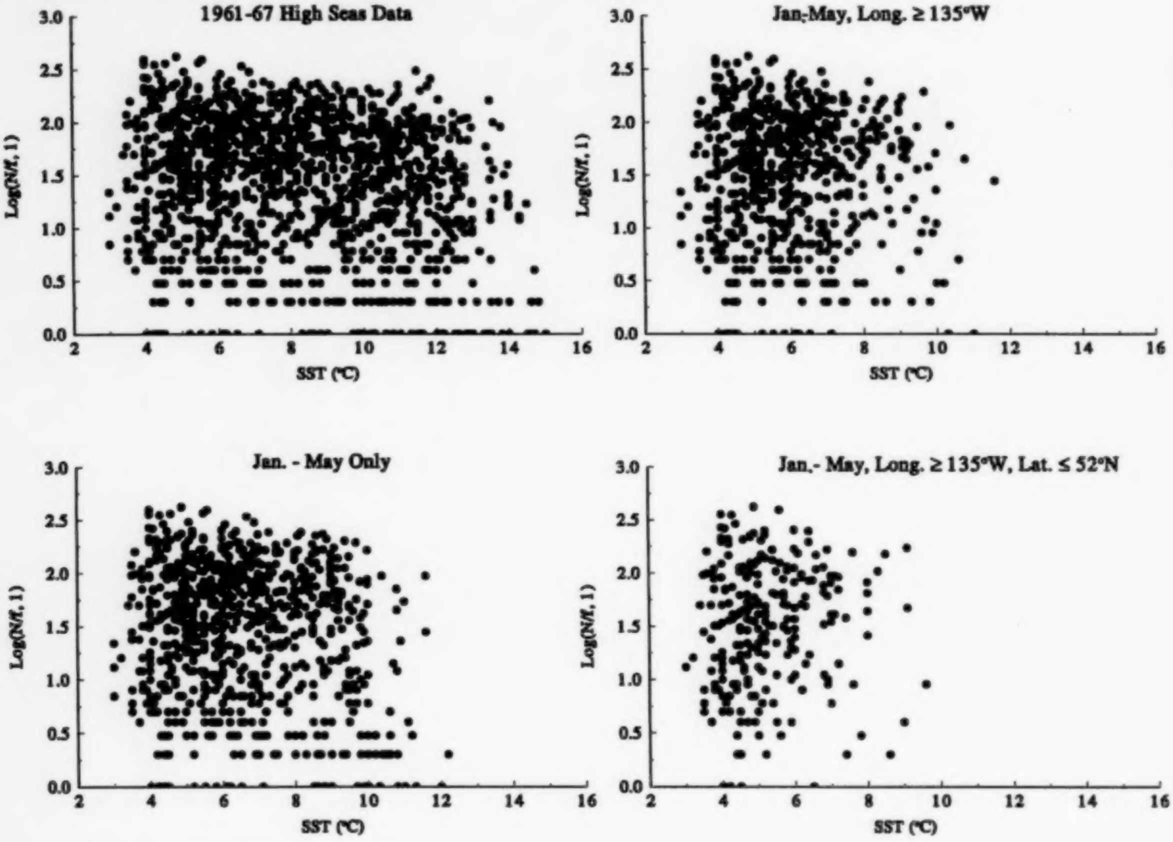
Fig. 8. Comparison of total salmonid catch versus temperature for the *R/V Tinro* and the 1987, 1988, and 1990 *W.E. Ricker* cruises. Confidence intervals on the *R/V W.E. Ricker* catches were calculated as in Fig. 7 but dividing the gear length (in metres) by 15 to calculate the degrees of freedom, *k*.



Sockeye caught in coastal waters have greater stomach content volumes than those caught in the offshore (Burgner 1991), and therefore growth rates in coastal waters should be maximized at higher temperatures than would be the case in offshore waters. As Pacific salmon are in fact found in much warmer surface waters in the productive coastal regions, the observed differences are consistent with the different levels of food availability on- and off-shelf.

Defining the limits to distribution can depend on the gear used, because more efficient gear may capture salmon in areas where none are captured with another gear. However, the good agreement between data obtained using gillnet, longline, and trawl gear suggests that the very sharp drop in abundance at 10–11°C is unlikely to be an artifact.

Fig. 9. Salmonid catch versus temperature for the 1961–1967 Canadian and U.S. High Seas salmon research cruises (Turner and Aro 1968). CPUE is numbers caught per unit hachi of surface longline. (A) Total data (all stations). (B) January to May only. (C) January–May stations taken in the offshore (west of 135°W). (D) January–May offshore stations taken in the south-central Gulf of Alaska (defined as south of 52°N).



Salmon are occasionally encountered at high temperatures in offshore regions of the North Pacific (particularly in summer, when most high seas salmon research takes place). This has raised concerns about the possible impact of high seas driftnet fisheries for squid on salmon (Burgner et al. 1982; Burgner and Meyer 1983; Takagi 1983; Walker and Burgner 1985; Dahlberg and Sigler 1987; Harris and Kautsky 1987). However, in large part these studies focused on identifying the occasional catch of salmon in anomalous conditions. As these incidents are by definition sporadic or rare, it is important that a statistical model be used to correctly take into account the occurrence of such observations. The use of the thermal range is a poor choice for defining the distribution of salmon because it is dependent on sample size and is influenced by rare excursions into extreme temperatures (Magnuson et al. 1979).

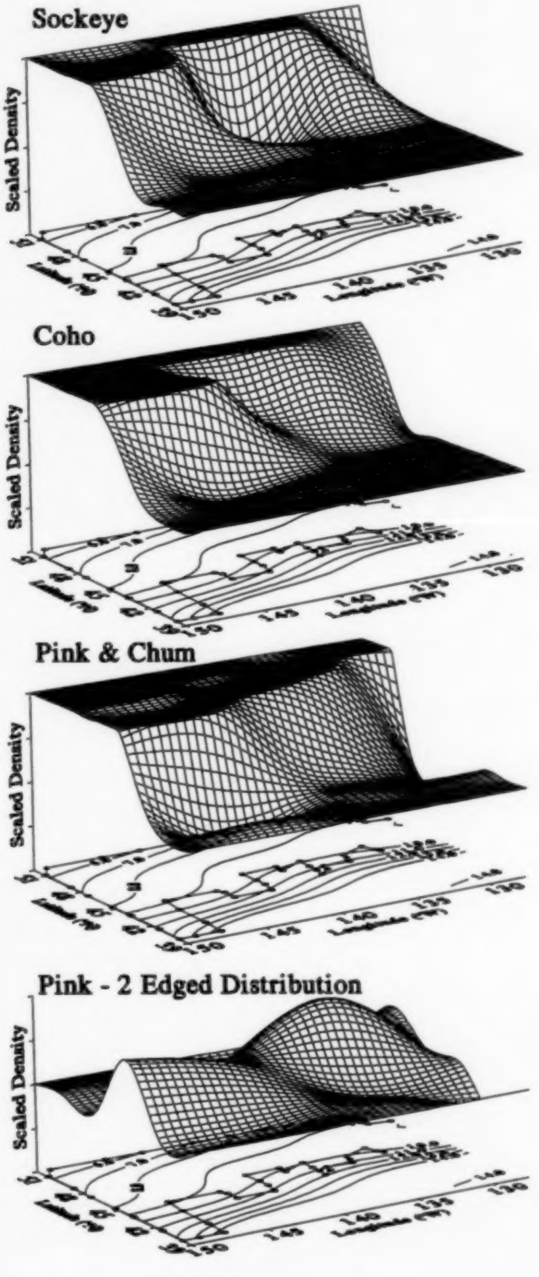
Nevertheless, both juvenile and maturing salmon are clearly found at higher temperatures in coastal and offshore waters than were observed during our surveys, and lethal limits for Pacific salmon exceed 20°C, irrespective of acclimation temperature (Brett 1956). Thus it is clear that temperature alone does not always determine the southern limit of the salmon distribution. However, the remarkably sharp response to temperature, and the low temperature at which this response occurs, suggest that the life history factors controlling the oceanic distribution of Pacific salmon probably play important roles in shaping the evolution of Pacific salmon.

Conclusions

The extremely sharp threshold response to temperature that we have identified in the North Pacific suggests that changes in ocean temperatures could have a profound effect on the dynamics of salmon production. A clearer insight into the influence of temperature on distribution will be important to understanding the impact of either physical oceanographic changes that may occur as a result of climate change or biological changes that may occur as the result of ongoing efforts to further increase salmon populations.

We know very little of the extended period of life in the open ocean when the majority of growth occurs (Ricker 1962, 1975). If regions of optimal temperature were to shrink because of climate warming, or if increasing salmon

Fig. 10. The predicted geographic distribution of salmon in April 1990, based on the *R/V Tinro* data. Contour plots show the predicted temperature field, while the surface plot shows the predicted mean response of salmon abundance to temperature, using the parameter estimates reported in Tables 2 and 3. Note the sharp drop in abundance well to the north of both the Transition zone and the Subarctic boundary. Fine details in the abundance surfaces are dependent on the accuracy of the interpolation of the temperature field.



Sockeye

Coho

Pink & Chum

Pink - 2 Edged Distribution

population sizes were to result in reduced per capita rations, behavioural thermoregulation could lead to salmon being restricted to a smaller part of the subarctic Pacific. Any existing trophic competition for food on the high seas would then be exacerbated by the higher densities that would result and lead to further contraction, a positive feedback. The potential for trophic competition has been examined elsewhere (Brodeur 1990; Welch and Parsons 1993), and some empirical evidence for density-dependent growth rates or species interactions on the high seas already exists (Krogius 1960; Sano 1963; Ogura et al. 1990; Ishida et al. 1993).

Conversely, if the forage base was to change, salmon distributions could shift relative to the thermal regime in response to increased or decreased ration sizes. Brett et al's (1969) studies indicate that behavioural thermoregulation could shift the upper thermal limit for juvenile sockeye from 8 to 12°C in response to a doubling of ration size from 3 to 6% of body weight·day^{-1}. A doubling of zooplankton and salmon biomass levels between the 1950s and 1980s in the Gulf of Alaska has been reported by Brodeur and Ware (1992, 1995). The effect of such changes in the forage base on salmon growth are now currently under study.

Our results provide some surprising predictions about the effect of the factors controlling salmon distributions on the high seas. If salmon are thermo regulating to maximize growth, then they should move into *colder* waters in the winter, when food resources decline, and then move south into *warmer* waters in the summer. A full mechanistic explanation of the high seas migrations and distribution of Pacific salmon is still premature; we believe, however, that such information will be essential to informed management of these resources in the future.

Acknowledgments

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An evaluation of light-mediated vertical migration of fish based on hydroacoustic analysis of the diel vertical movements of rainbow smelt (*Osmerus mordax*)

A.R. Appenzeller and W.C. Leggett

Abstract: We used hydroacoustics to examine diel changes in the vertical distributions of rainbow smelt, *Osmerus mordax*, in Lake Memphremagog, Quebec/Vermont. Our objective was to evaluate hypotheses linking diel vertical movements of fish with light levels. Smelt distributions were also monitored from June through October (1988 and 1990) to evaluate seasonal changes in their behavior. A strong relationship ($r^2 = 0.83$) between ambient light intensities and the upper fish layer in the water column was observed. Fish depth was also related to the depth of the thermocline during the night and when surface water temperatures were >18°C. The most characteristic feature was the strong avoidance of light levels >0.1 μW/cm^2. However, we found considerable variation in lower light levels experienced by the whole fish population. The results suggest that existing models of anti-predation behavior relating light and fish depth are consistent, with some limitations, with patterns of diel vertical migration in rainbow smelt.

Résumé : Nous avons eu recours à des moyens hydroacoustiques pour examiner les changements nycthéméraux de la distribution verticale de l'éperlan arc-en-ciel, *Osmerus mordax*, dans le lac Memphremagog, au Québec et au Vermont. Notre objectif était d'évaluer des hypothèses liant à l'éclairement les mouvements verticaux nycthéméraux des poissons. Nous avons aussi surveillé la répartition des éperlans de juin à octobre (1988 et 1990) pour évaluer les changements saisonniers de leur comportement. Nous avons observé une forte relation ($r^2 = 0,83$) entre l'éclairement et la présence des poissons dans la couche supérieure de la colonne d'eau. La profondeur des poissons était aussi liée à la profondeur de la thermocline pendant la nuit et quand la température superficielle de l'eau était >18°C. Le trait le plus caractéristique était un évitement marqué des éclairements >0,1 μW/cm^2. Toutefois, nous avons trouvé une variation considérable dans le niveau inférieur d'éclairement pour l'ensemble de la population de poissons. Les résultats semblent indiquer que les modèles actuels de comportement anti-prédateur qui mettent en relation l'éclairement et la profondeur des poissons correspondent, avec certaines limitations, aux profils de la migration verticale nycthémérale chez l'éperlan arc-en-ciel.

[Traduit par la Rédaction]

The occurrence of diel vertical migrations has been reported for a wide range of zooplankton and fish taxa (Woodhead 1966; Blaxter 1975; Lampert 1989). While no unifying theory explaining such behavior exists, a number of hypotheses have been advanced which relate to both its ultimate and proximate causes (MacLaren 1963; Brett 1971; Dembinski 1971; Blaxter 1980; Janssen and Brandt 1980; Wurtsbaugh and Li 1985; Geller 1986). The predator avoidance hypothesis suggests that the avoidance of light-related mortality is the ultimate cause of such behavior (Eggers 1978; Stich and Lampert 1981; Gliwitz 1986; Gabriel and Thomas 1988; Lampert 1989). Clark and Levy (1988) have extended this theory for juvenile fishes and have incorporated trade-offs between the enhancement of foraging success and predator avoidance of vertical migrators. Their model is based on the assumption that vertical migrators, in this case juvenile fish, feed mostly during crepuscular hours to avoid visual detection by their prospective predators; they called this period the antipredation window. There are many observations that show that this model could be applicable to different migrating fish

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species. In fish, diel movements have been observed frequently to correspond to the depth of equal light intensities (isolumes) (Clarke and Backus 1964; Boden and Kampa 1967; Blaxter 1975; Huse and Holm 1993). Moreover, the daylight depth of occurrence of several fish species has been linked to water transparency, supporting the hypothesis that light intensities are an important regulator of the depth of fish (Woodhead 1966; Dickson 1972; Isaacs et al. 1974; Kampa 1976; Levy 1990*b*). Additional evidence for light-mediated changes in the vertical distribution of fishes has been provided by the observation that fish move to shallower waters during solar eclipses (Schüler 1954; Backus et al. 1965; Kampa 1976).

Melnikow et al. (1981) proposed that if the light preferences of the animal in question were known, its depth distribution could be predicted from a knowledge of factors determining illumination levels. Despite ample evidence for light-mediated vertical migrations of fishes, it is also clear that the specific patterns reported are highly variable relative to ambient light levels, change over the season, and can also be reversed with respect to light (Brawn 1960; Woodhead 1966; Turuk 1973; Blaxter and Parish 1965; Levy 1990*a*). Several authors (Blaxter 1975; Heath et al. 1988; Neilson and Perry 1990) commenting on this variety of behaviors, have observed that most reports of diel vertical migrations in fishes are circumstantial, being based primarily on qualitative estimates of the depth distribution of the populations in question, and further, that light-regulating mechanisms have not been substantiated with rigorous tests involving in situ measurements of the variables in question.

The question therefore remains whether light is the primary regulator of diel vertical migrations in fishes, and whether it is possible to develop empirical models that reliably predict the depth distribution of fish on a diel and (or) seasonal basis. Hydroacoustic techniques provide the means necessary to quantitatively assess the vertical distribution of fishes with high resolution, and thus to evaluate more precisely the role of light as a regulator of diel behaviors (Huse and Holm 1993).

Our objectives in this study were therefore to (*i*) quantitatively assess the pattern of vertical migration in a species known to be light sensitive; (*ii*) to evaluate the depth-specific in situ changes in light levels associated with these movements; and (*iii*) to discuss the results in relation to light-mediated models of vertical migration in fishes (Melnikov et al. 1981; Clark and Levy 1988).

Methods

Study site and hydroacoustic methods

We chose rainbow smelt (*Osmerus mordax*) as our experimental species. Smelt have been described as light sensitive (Ferguson 1965) and are known to exhibit diel vertical migrations, moving to deeper waters during the day and to shallower waters at night (Dembinski 1971; Argyle 1982; Heist and Swenson 1983; Burczinsky et al. 1987). We conducted the study in the central basin of Lake Memphremagog (Quebec/Vermont). Smelt dominate the pelagic fish community of this lake. The mean depth of the study basin is 50.9 m (max. depth 105 m). This basin is an elongated (15 × 2 km), deep trench with very steep sides. The depth and the shoreline steepness provide almost unlimited and continuous access to smelt via hydroacoustic sampling (Appenzeller and Leggett 1992).

A 2-km transect oriented perpendicular to the shore was sampled 116 times during 1988 and 67 times during 1990. In 1988 we sampled from June to October and data were recorded on 29 different days; in 1990 we sampled on 5 days in July and August. We used a 120 kHz Biosonics model 105 dual-beam echosounder (beam widths 11 and 25 degrees) to quantify fish in the study area. The system was mounted in a 5 m long boat. Speed during all surveys was maintained at 8.5 km/h. All lights were extinguished during night sampling to avoid attraction and (or) disturbance of the fish being surveyed. Fish biomass and average fish size were quantified by 20-log R (echointegration) and 40-log R (dual beam) amplification of echosignals, respectively. Fish abundance was estimated by dividing the total acoustic biomass measurements by the in situ average target strength. The vertical distribution of fishes was averaged over the full transect for each depth zone studied. Estimates of fish abundance (no./m^3) were subdivided into 1-m depth windows from depths of 2–60 m. We found fish abundance to be very low at depths >60 m.

We conducted a series of trials to calibrate and identify echo signals. We ensonified targets at night at fixed locations in the lake, then sampled immediately with a 9-m^2 vertical haul net. Only smelt were caught. The dominant (>80%) length class was 12 cm (SD 1.8) and a less abundant size class averaging 5 cm standard length was also captured. From July through August 1990 we restricted our acoustic analyses to the depth range 10–60 m, because waters <10 m were inhabited exclusively by large numbers of small (15–30 mm) nonmigrating young-of-the-year smelt.

Variables

Time of day

The time of the beginning and end of each transect was transformed to local time by calibrating to the time of the sun's zenith at Lake Memphremagog (local noon).

Light

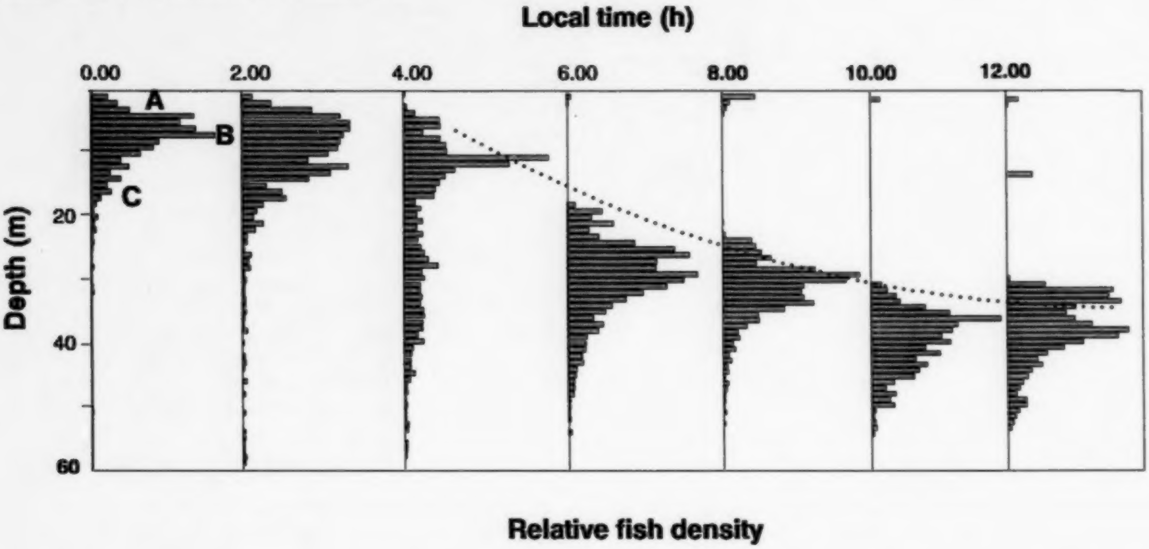
Light intensity measurements were made with an underwater irradiameter (Kahl Scientific Instrument Corp., model 268 wa 310). The instrument had a measurement range of 0.1–10000 μW/cm^2. This unit allowed us to measure ambient light on board the survey vessel and to depths of 30 m in the lake. Ambient light readings were averaged over the entire transect for statistical analyses. To derive illumination levels at the depths where fish occurred we empirically modeled the reduction of ambient light levels as a function of (*i*) light transmission through the water surface and (*ii*) light attenuation in the water column with depth. Light levels in the water column were highly correlated with depth ($n = 28$; $r^2 = 0.99$) according to the general equation

$$[1] \quad \ln I(d) = \ln I(s) - \epsilon \cdot D$$

whereby $I(d)$ is the light intensity at depth d in metres, $I(s)$ is the light intensity below the water surface, ϵ is the attenuation coefficient, and D is the depth in metres.

The combined attenuation and transmission coefficients were used to calculate light levels at depth from our ambient

Fig. 1. Acoustic data on the vertical migration of smelt in Lake Memphremagog. Fish density measurements are averaged from an entire transect across the lake subdivided into 1-m depth intervals. Fish densities were highest in the upper water column at night (0:00–4:00 h) and shifted subsequently into deeper water layers between 4:00–10:00 h. An isolume of ca. 0.1 μW/cm^2 (dotted line) is shown in the water column. (A) Upper 95th percentile fish depth. (B) Mode of the fish depth (maximum). (C) Lower 95th percentile depth.



light readings. For comparison with illumination levels given in the literature that are reported in units other than μW/cm^2 we used conversion factors given in Brock (1981).

Temperature
Temperature profiles were conducted weekly at 1-m intervals to a depth of 40 m. These were repeated on both the east and west ends of the transect to account for possible differences in the vertical profile caused by internal waves.

Vertical fish distribution
We used the following statistics to characterize the observed vertical fish depth distributions: the upper 95th percentile, the mode, and the lower 95th percentile of the entire fish layers between 2 and 60 m depth (Fig. 1). These measures indicate (*i*) the depth at which the maximum fish density occurred (the mode), (*ii*) the approximate range of vertical dispersion (given by the upper and lower 95th percentiles). This range describes the behavior of the majority of the fish population (90%) which was contained within these depths.

Results

Smelt in Lake Memphremagog exhibited a strong vertical migration. They were typically concentrated in the upper 10–15 m during nighttime hours, and migrated to deeper waters during daylight. Typically, peaks in daytime distributions occurred at 30–40 m (Fig. 1). Aggregation patterns also varied between day and night. Smelt were dispersed during the night and formed dense schools during daylight hours. This change in aggregation pattern was coincident with their vertical displacement (Appenzeller and Leggett 1992). The pattern of vertical migration was modified seasonally by water temperature. When epilimnetic temperatures exceeded 18°C, smelt aggregated just below the thermocline depth during nighttime hours.

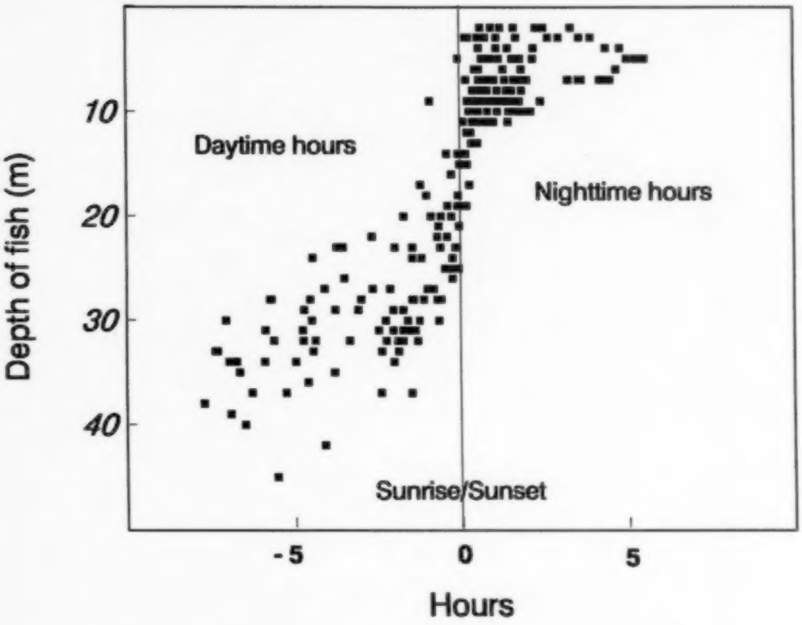
Local time and the depth of fish in relation to sunset and sunrise

To describe the diel vertical displacement of smelt we plotted the depth of fish (mode) in relation to local hours from sunset or sunrise at lake Memphremagog (Fig. 2). This graph shows that during the day the layer of maximum fish density was located mostly in water depths between 30 and 40 m. At sunset fish moved into the upper water column and stayed in the upper 10 m during the night. Figure 1 also shows the variability in fish depths in relation to local time, possibly associated with seasonal changes in environmental variables. However, the sunset/sunrise midpoint in fish depths (ca. 15m) is fairly stable during the whole season.

Ambient light and fish depth

Fish depth during daylight hours was linearly related to the log of ambient light. These relationships were highly significant for the upper 95th percentile depth ($r^2 = 0.83$) and the modal depth ($r^2 = 0.78$). However, the lower 95th percentile depth was only weakly related to light ($r^2 = 0.14$; Fig. 3). We obtained an improved fit to the data on the upper 95th percentile by applying a nonlinear model with two lines intersecting ($r^2 = 0.87$; $p < 0.01$; Fig. 3). This improved model suggests that the strong linear relationship between ambient light and the upper 95th percentile depth of fish was dampened at depths <10 m during crepuscular hours.

Fig. 2. Relationship between the mode (maximum fish density) of the vertical smelt distribution and local time of day (relative to sunrise/sunset). Each point represents data from an entire transect (n = 228).



Light levels at the depth of fish

Illumination levels at the depth where fish were located averaged 0.11 μW/cm^2 (SD 0.42) at the depth of the upper 95th percentile, 0.012 μW/cm^2 (SD 0.03) at the modal depth, and 9×10^{-6} μW/cm^2 (SD 4×10^{-5}) at the lower boundary of the fish distribution. These illumination levels exhibited trends with local time: at noon fish stayed at lower illumination levels than during the hours close to either sunset or sunrise (Fig. 4). This pattern was clearly visible for the upper boundary of the fish layer and could be interpreted as fish being more risk prone close to the crepuscular hours when they feed. The illumination level at the lower boundary of fish (Fig. 4), however, was almost stable during daylight hours, and even dropped considerably after sunrise/sunset. This pattern in fish vertical movements has a simple explanation: during crepuscular hours the deeper fish stopped their upward movements after sunset, and vice versa. Similarly, after sunset, as they reached the upper warm water layers, the fish in the upper boundary stopped migrating as well.

The influence of the temperature of the mixed layer on the vertical migration of smelt

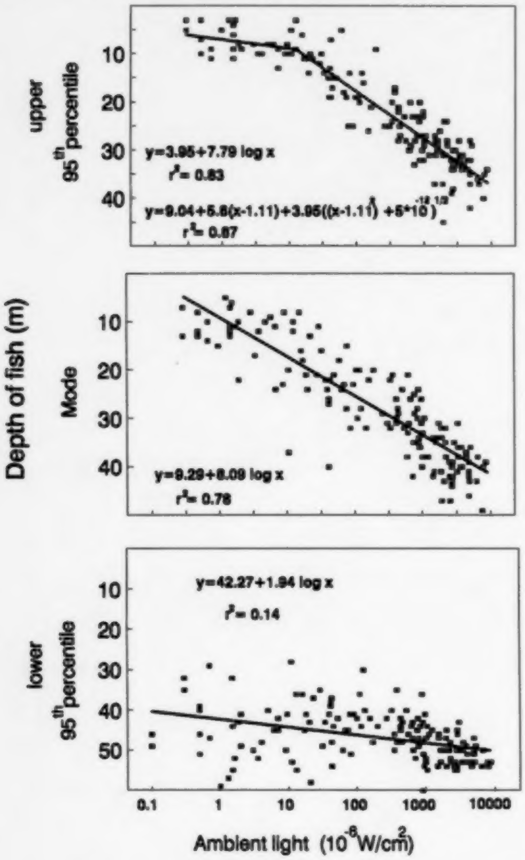
Mixed layer temperatures increased from 11 to 25°C between early June and mid-August, and declined thereafter to 10°C in early October. The depth of the thermocline increased steadily with season. There was evidence for an influence of this seasonal change in temperatures on the vertical distribution of smelt during nighttime at the upper boundary of the fish layer (Fig. 5). The effect of thermocline depth on fish depth was strong when epilimnetic waters exceeded 18°C. Under those conditions the upper boundary of fish depth was related to the depth of the thermocline ($n = 69$; $r^2 = 0.53$). In early spring and late fall, when smelt were not constrained by epilimnetic temperatures >18°C, they entered near-surface waters at night, where they then were less vulnerable to our hydroacoustic gear. During these surveys the echo-integration values dropped considerably at night and smelt were readily observed at the water surface.

Discussion

Our results are consistent with previous observations (Ferguson 1965) that smelt are "light sensitive" and avoid waters with high light levels. Daytime illumination levels in surface waters can exceed several thousand μW/cm^2. However, the average illumination level at the upper boundary of smelt distribution in Lake Memphremagog was 0.12 μW/cm^2 (SD 0.42), and the light level associated with the depth of maximum fish abundance was 0.012 μW/cm^2 (SD 0.03). By way of comparison, Heist and Swenson (1983) reported maximum smelt densities at illumination levels of <0.001 μW/cm^2 in Lake Superior, and Dembinski (1971) reported that smelt schools in Polish lakes occurred at light levels ranging from 0.004 to 20 μW/cm^2. These data suggest a strong and persistent tendency of smelt to avoid light levels >20 μW/cm^2. The variation in the light levels associated with smelt distributions in these studies could be the result of differences in the calibration and sensitivity of the light sensors used.

Our data clearly show that moments of the vertical distributions of smelt were related strongly and significantly

Fig. 3. The relationships between fish depth and ambient light. For the upper 95th percentile depth a model with two intersecting lines was applied. Linear regression models were fitted for the mode and lower 95th percentile. Data are based on n = 148 acoustic transects.



to ambient light levels. The changes in fish depth in relation to light levels we observed could be interpreted as "fish following optimal light levels" (Boden and Kampa 1967; Blaxter 1975; Melnikov et al. 1981). However, the range in the fish depth distributions provides strong indications that these fish exhibit negative phototactic behavior. This is suggested by the following (*i*) The shape of the smelt depth distributions that showed a strong asymmetry, with the mode of the distribution being closer to the upper 95th percentile depth. This skewness was repeatedly observed in data from single transects (Fig. 1). (*ii*) The ranges of the fish depth distribution, measured by the upper and lower 95th percentiles, differed considerably in their predictability in relation to ambient light (Fig. 3).

These results show that the vertical depth range of fish cannot be predicted consistently from a knowledge of the upper and lower boundaries of fish light preferences as suggested by Melnikov et al. (1981).

This is only possible for the upper boundary and the depth of maximum fish abundance. The impact of light on the vertical distribution of smelt can be imagined as a compression of the available space in the vertical dimension from above, whereby fish aggregate at the upper boundary of their depth range at a fixed light level and respond strongly to changes in the depth of this illumination threshold. We conclude therefore that the avoidance of high illumination levels rather than the seeking of a light optimum is the dominant proximate cause for the vertical distribution patterns we observed.

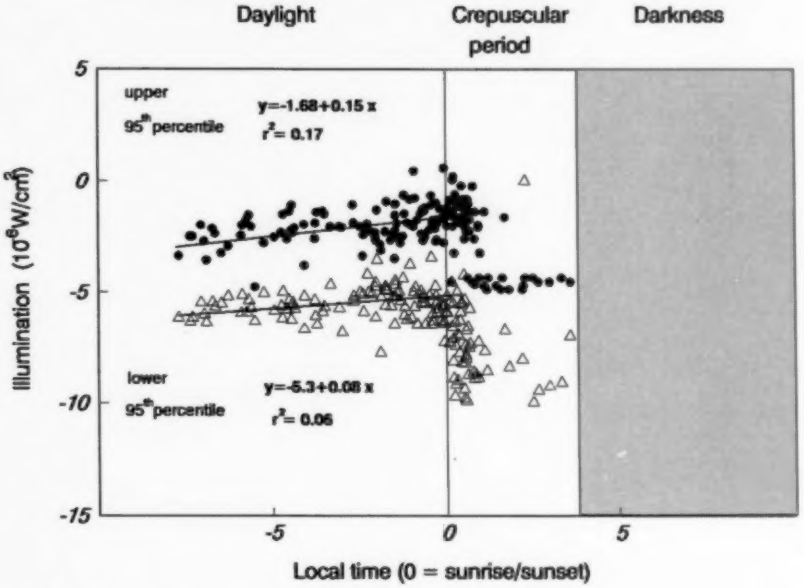
In comparison, Heath et al. (1988) studied the vertical distribution of larval herring, *Clupea harengus*, and found that the distribution of fish deep in the water column was more strongly related to ambient light than was the distribution of fish in the near-surface waters where light levels were highest. They interpreted this pattern to reflect positive phototactic behavior by herring larvae, leading to aggregation in the upper water layers during the day.

Our results and observations on smelt vertical distribution support the hypothesis that such patterns in fish possibly evolved as a consequence of light-related mortality, and that it has an anti-predation function (Iwasa 1982; Gliwitz 1986; Gabriel and Thomas 1988; Lampert 1989; Gliwitz and Jachner 1992). A basic assumption of this hypothesis is that predation and the reactive distances of predators that search visually increase with light intensity (Vinyard and O'Brien 1976; Clark and Levy 1988). Smelt, a small pelagic fish, is commonly preyed upon by large visually oriented predators (Evan and Loftus 1987). Smelt predators in Lake Memphremagog such as lake trout (*Salvelinus namaycush*) were frequently observed 10 m above the depth where smelt occurred during the daytime. These piscivorous fish feed mostly during twilight hours when smelt move vertically in the water column. This pattern of smelt vertical migration is consistent with Clark and Levy's (1988) view that vertically migrating juvenile fish possibly optimize a trade-off between faster growth and higher mortality by feeding during twilight hours, when the predation risk is reduced. Our study also showed that during crepuscular hours smelt were at slightly higher illumination levels. This observation could be interpreted as an example of more risk-prone behavior of smelt during the twilight period when they are still able to feed on zooplankton, while their own predators could have difficulty adapting to decreasing illumination levels (Pitcher and Turner 1986).

The antipredation window model of Clark and Levy (1988), therefore, could be applicable to the more general case of small pelagic fishes, such as smelt, that feed mostly during crepuscular hours in the pelagic zone (Narver 1970; Bohl 1980; Levy 1987 and 1990*b*). However, an important difference must be noted. In the case of smelt, only 1-year-old fish and older migrate vertically. Young-of-the-year larvae and juveniles stay, according to our own observations, in upper water layers during both day and night, being thus exposed to visual predators during sunny conditions. This change in habitat during the life history of smelt is related to the differences in thermal adaptation with age. One-year-old and older fish are adapted to colder waters than young-of-the-year, with an upper limit of 16–18°C (Evans and Loftus 1987; Burczinsky et al. 1987; Gliwicz and Jachner 1992).

We found that temperature had an important effect on the

Fig. 4. Relationship between the light at fish depth (log $\mu W/cm^2$) and local time of day (relative to sunrise/sunset). ●, upper 95th percentile fish depth; △, illumination levels at the depth of the lower boundary of fish depth. The light levels for the upper boundary increased towards sunset providing evidence of possible risk-prone behavior of fish during these hours. During crepuscular hours the sudden drop in light levels was related to a stop in the vertical migration of fish as they reached mixed layer temperatures. The lower boundary of fish depth did not move higher during these hours, and thus their illumination levels drop consequently.



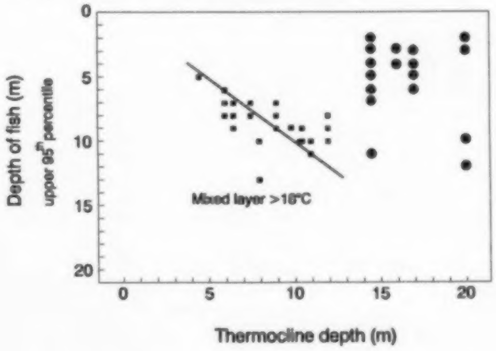
vertical distribution of smelt at night during months when epilimnetic temperatures were >18°C. Under these conditions, fish aggregated near the base of the thermocline. This pattern indicates a change in the dominant limiting factors at night with the light barrier absent. Such aggregations in the metalimnetic waters close to the thermocline have also been observed for other vertically migrating fish (Narver 1970; Hamrin 1986; Burczinsky et al. 1987; Wurtsbaugh and Neverman 1988; Levy 1990*b*). At night, therefore, temperature may act as a major sensory cue for fish (Levy 1987), with light prevailing during the day.

Practical applications of vertical migration of fish species

A knowledge of the patterns of vertical migration of fish can have considerable practical application in hydroacoustic fish surveys (Shotton and Bazigos 1984). Our results suggest that during times of the year when surface temperatures are cold, (in this case <18°C) the upper boundary of smelt distributions approach the surface where fish become inaccessible to the acoustic beam. Hence, a considerable bias will occur if hydracoustic surveys are conducted at night under such conditions. In our study, estimates of fish abundance declined by up to 50% at such times.

In shallow lakes, or coastal oceanic areas, where light illumination near the bottom approaches or exceeds levels typically avoided by the species in question, fish may be located very close to the bottom during the day. Under such conditions quantification by acoustic methods may be impossible during daylight hours because fish and bottom signals cannot be reliably separated. Under such

Fig. 5. Relationship between thermocline depth and the depth of fish at Lake Memphremagog during the night. Fish at the upper boundary of the vertical depth range stopped migrating when the mixed layer temperature was >18°C. From this behavior follows a tight relationship of fish depth and thermocline depth during the summer when mixed layer temperatures exceeded 18°C (□, regression line). However, when this temperature dropped below 18°C there was no such relationship (●).



situations, only night surveys conducted subsequent to the development of a well-defined thermocline and unsuitable epilimnetic temperatures would yield reliable estimates of fish biomass. In short, the seasonal and diel time window during which accurate hydroacoustic biomass or abundance estimates can be made may be short, and the optimal time for such surveys may be species and (or) site specific.

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Effects of 3,3′,4,4′-tetrachlorobiphenyl on the dynamics of vitamin A in brook trout (*Salvelinus fontinalis*) and intestinal retinoid concentrations in lake sturgeon (*Acipenser fulvescens*)

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Abstract: A single i.p. injection of 5 μg 3,3′,4,4′-tetrachlorobiphenyl (TCBP)/g body mass in adult brook trout (*Salvelinus fontinalis*) resulted in decreased ($p < 0.0001$) growth rate despite pair feeding. Plasma retinol decreased ($p < 0.0037$). Intestinal retinyl palmitate (RP) and 3,4-dehydroretinyl palmitate (DRP) concentrations decreased in TCBP-injected males (RP, $p < 0.0143$; DRP, $p < 0.0009$), whereas retinoid levels did not decrease significantly in TCBP-injected females. The RP:DRP ratio in trout liver increased ($p < 0.0001$). These results suggested that DRP is more sensitive than RP to the effects of TCBP. No significant differences in ovarian retinoids occurred in post-spawning trout. Field validation of the effects on intestinal retinoids was conducted with lake sturgeon (*Acipenser fulvescens*) caught at a contaminated site on the Des Prairies River near Montréal (St. Lawrence River population) and reference sturgeon taken from a site near the origin of the Ottawa River in LaVerendrye Park. Intestinal retinoid concentrations were lower (RP, $p < 0.0008$; DRP, $p < 0.0004$) in the St. Lawrence River sturgeon. Our results demonstrate that a coplanar PCB is capable of altering vitamin A dynamics in several tissues and organs, and may cause a lowering of retinoids in the intestine.

Résumé : Une injection i.p. de 3,3′,4,4′-tétrachlorobiphényle (TCBP), 5 μg/g de poids corporel, a entraîné une réduction ($p < 0.0001$) du taux de croissance chez l'omble de fontaine (*Salvelinus fontinalis*), malgré le fait que les groupes témoins et traités avaient consommé la même quantité de nourriture par p.c. Le rétinol plasmatique a diminué ($p < 0{,}0037$). Les niveaux de palmitate de rétinol (RP) et de 3,4-déshydrorétinyl palmitate (DRP) dans les intestins des mâles traités ont diminué (RP, $p < 0{,}0143$; DRP, $p < 0{,}0009$), contrairement aux femelles traitées dont les niveaux de rétinoïdes n'ont pas diminué. Le rapport RP:DRP dans le foie a augmenté ($p < 0{,}0001$). Ces résultats suggèrent que le DRP est plus sensible que le RP quant aux effets du TCBP. Les concentrations des rétinoïdes ovariens n'ont pas été affectées. Les effets du TCBP sur les rétinoïdes intestinaux ont fait l'objet d'une validation sur le terrain avec les esturgeons jaunes (*Acipenser fulvescens*) capturés dans un site contaminé de la rivière des Prairies, près de Montréal (population du fleuve Saint-Laurent), et dans un site référence sur la rivière des Outaouais, dans le parc LaVérendrye. Les concentrations des rétinoïdes intestinaux étaient plus faibles (RP, $p < 0{,}0008$; DRP, $p < 0{,}0004$) chez les esturgeons du site contaminé. Nos résultats démontrent la capacité d'un BPC coplane d'affecter la dynamique de la vitamine A dans plusieurs tissus et organes, entre autres les intestins.

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Introduction

Vertebrate organisms tend to accumulate retinoid (vitamin A) stores that may be utilized according to physiological needs or during periods of inadequate nutritional intake. We recently found extremely low liver retinoid stores in white sucker, *Catostomus commersoni*, and lake sturgeon, *Acipenser fulvescens*, captured near Montreal (Spear et al. 1992; Branchaud et al. 1995). These results are reminiscent of the extremely low liver retinoid stores in piscivorous birds nesting in heavily contaminated areas of the Great Lakes (Spear et al. 1986, 1992). In addition to low retinoid concentrations, the Montreal fishes have an elevated prevalence of embryonic deformities, a low frequency of running-ripe females (or females in which egg maturation could be induced by hormone injections) and increased cytochrome P-450IA1-dependent ethoxyresorufin-deethylase (EROD) activity (Branchaud et al. 1995; P.A. Spear and A. Branchaud, unpublished data). Histopathologic lesions were detected in the Montreal sturgeon (Rousseaux et al. 1995).

Although many factors may have contributed to the condition of the Montreal fishes, the observations are consistent with exposure to polycyclic aromatic hydrocarbons (PAHs) or certain polyhalogenated aromatic hydrocarbons such as the coplanar PCBs. In the case of both sucker and sturgeon caught near Montreal, 3,3′,4,4′-tetrachlorobiphenyl (TCBP) was the predominant coplanar PCB in the eggs (P.A. Spear, A. Branchaud, and B. Bush, unpublished data). Eggs of the lake sturgeon caught in the Montreal area contained up to 1.1 μg TCBP/g, while the sum of coplanar biphenyls reached 2.2 μg/g in the eggs of one female. Elsewhere, TCBP concentrations in the striped mullet (*Mugil cephalus*) were reported to be 4.8 ng/g (Tanabe et al. 1987) and carp (*Cyprinus carpio*) sampled from the Ohio River contained 0.11 μg TCBP/g (Huckins et al. 1980). Rainbow trout (*Oncorhynchus mykiss*) exposed to TCBP in the water accumulated up to 12.0 μg/g in body tissues (Stalling et al. 1979).

Coplanar biphenyls are among the most deleterious polychlorinated biphenyls (PCBs) having toxicological properties similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Parkinson et al. 1983). They are thought to be the principal contributors to the toxicity of mixtures involving coplanar PCBs, dioxins, and furans in aquatic ecosystems (Tanabe et al. 1987; Niimi and Oliver 1989; Jones et al. 1993). Diverse coplanar PCBs decrease hepatic vitamin A stores in the rat (Azais et al. 1987; Spear et al. 1988), mouse (Brouwer et al. 1985), and ring dove (Spear et al. 1986, 1989). Lake trout, *Salvelinus namaycush*, injected with the coplanar PCB, 3,3′,4,4′,5-pentachlorobiphenyl, had dramatically decreased liver stores of vitamin A (Palace and Brown 1994). The biological effects of PCBs in laboratory rodents resemble those seen during vitamin A deficiency (Innami et al. 1974; Brouwer and Van Den Berg 1984; Azais et al. 1987). In the case of humans where a relatively complete set of observations is available, the major effects common to PCB–furan–dioxin toxicity and vitamin A imbalances include reproductive inhibition, teratogenicity, growth inhibition, body mass loss, various dermal disorders (e.g., hyperkeratosis, chloracne-like condition), immunosuppression, susceptibility to infections, and susceptibility to cancers (Parkinson and Safe 1981; Safe 1984; Kamm et al. 1984; Underwood 1984; Spear 1985). Such observations have led to the hypothesis that imbalances in vitamin A homeostasis may be an integral part of the mechanism of action of these environmental contaminants.

Coplanar PCBs may affect retinoid dynamics through the induction of liver enzymes (e.g., cytochrome P-450IA1; UDP-glucuronyltransferase), which catabolize the retinoids (Spear et al. 1988; Bank et al. 1989). Additionally, coplanar PCBs may alter circulating levels of retinol in the blood possibly by interference with the retinol–retinol-binding protein–transthyretin (retinol–RBP–TTR) complex (Brouwer and Van Den Berg 1986) or through decreased dietary supply related to loss of appetite (Spear et al. 1994). The alteration of blood retinol concentrations may affect retinoid levels in specific organs upon exposure to coplanar PCBs.

Little is known of the dynamics of retinoids in different organs of fish under normal conditions or in the case of fish exposed to coplanar PCBs. The first aim of the present study, therefore, was to test the hypothesis that TCBP alters retinoid concentrations in various tissues in brook trout (*Salvelinus fontinalis*). The second objective was to examine the effects of TCBP on growth and appetite of brook trout as previous studies with rodents had demonstrated indirect effects of coplanar PCBs on tissue retinoid concentrations through transient loss of appetite (Spear et al. 1994). Single i.p. injections of 5 μg TCBP/g were used as this dose level approximates the coplanar biphenyl concentrations detected in sturgeon eggs. Samples of lake sturgeon were also analyzed to test the hypothesis that effects observed in the laboratory may also be occurring in the environment.

Material and methods

Experimental fish and holding conditions

Fourty-eight sexually mature brook trout (length, 23.0–27.0 cm; weight, 160–277 g; age, 2 years) were purchased from Pisciculture Bélanger Inc., Labelle, Québec, in January 1993 and transported to the Ecotoxicology Laboratory at Concordia University. The brook trout had spawned in September 1992 and were in an early phase of gonad maturation. Fish were allowed to acclimate to laboratory conditions for 3 weeks. They were held in a 600-L fiberglass aquarium (Frigid Unit, Inc., 3214 Sylvania Ave., Toledo, Ohio) supplied with a flow of 10 L/min of city of Montreal tap water. The water having a $CaCO_3$ hardness of 133 mg/L was treated with activated charcoal, aerated and maintained at 9–13°C. Floating trout food (grower; 5 pt pellet size; Martin Mills Inc., Ste. Agathe, Québec) was given ad libitum and the photoperiod was 12 h light : 12 h dark. No mortality or infectious disease was observed during transport, acclimation, or experimental periods.

Following acclimation, individuals were randomly assigned to either control or exposed groups for the 8-week experiment; each group comprised two replicates, each of 12 fish. Controls received a single i.p. injection of corn oil (125 μL/100 g body mass (BM)) while exposed fish received a single i.p. injection of 5 μg TCBP/g BM in corn oil (TCBP purchased from Ultra Scientific, Kingstown, RI 02852). One fish was discarded after being weakened during handling.

Each replicate of 12 fish was held in a 200-L fiberglass tank with a 4–5 L/min water flow-through system that exceeded the recommended flow rates (Sprague 1973; Oikari et al. 1988). The controls were pair-fed because the TCBP-injected fish consumed less than controls. Each day, the food consumed by the exposed group was estimated by supplying a known amount of food and subtracting unconsumed food (estimated from the number of pellets remaining after 1 h). The following day, controls received the amount of food consumed by the exposed fish the previous day. All the food supplied to the control fish was consumed. Fish were weighed weekly (weeks 2–7). Tanks were siphoned daily before feeding to prevent the accumulation of organic materials.

Sampling of fish

Each trout was carefully taken from the tank, with minimal disturbance to other individuals and anesthetized with 1:1000 M.S. 222 (Aldrich Chemical Company, Inc., Milwaukee, WI 53233). Fish were quickly weighed, measured, and 1–1.5 mL blood samples were taken from the caudal vasculature, using heparinized 1-mL syringes (Becton Dickinson & Co., Butherford, NJ 07070) and held on ice. Twenty minutes after sampling, the blood was centrifuged (2 min; 15 000 × g) and the plasma stored at −80°C. Then, the fish was decapitated and liver, right gonad (middle part), head kidney (pronephros), spleen, and a 2 cm anterior segment of intestine were excised. Intestinal contents were removed by pressure applied with the blunt edge of a scalpel. All the tissues were immediately frozen in liquid nitrogen and stored at −80°C until analyzed. For a given fish, dissection was completed within 6 min (i.e., time between anaesthesia and tissue preservation, except plasma).

The condition factor (CF) was calculated from the total mass and the standard length according to the formula: $CF = [W\ (g)/L\ (cm)^3] \times 100$ (Oikari et al. 1988). Hepatosomatic index (HSI), gonadosomatic index (GSI), and splenosomatic index (SSI) were calculated as a ratio of the organ (liver, gonad, and spleen, respectively) fresh mass to total fresh mass of fish.

In the second experiment, adult lake sturgeon, *Acipenser fulvescens*, were caught with gill nets at a reference site near the origin of the Ottawa River in LaVerendrye Park ($N = 11$) and at a contaminated site on the Rivière des Prairies (confluent with the St. Lawrence River) near Montreal ($N = 9$). An anterior section of the intestine was excised, the lumen was cleared by rinsing with distilled water, the tissue was frozen on dry ice for transport, and stored at −80°C. Details of the field work and a full account of liver and egg RP and DRP concentrations, EROD induction, teratogenesis, histopathology, and PCB contamination are the subjects of future publications.

Retinoid analysis

Retinoids were analyzed by reverse-phase high performance liquid chromatography (HPLC). All-*trans*-retinol and all-*trans*-retinyl palmitate were purchased from Sigma Chemicals (St. Louis, Mo.). Authentic 3,4-dehydroretinol and 3,4-dehydroretinyl palmitate were synthesized in our laboratory following methods outlined in Barua and Olson (1984).

Plasma analysis for retinol (ROH) was modified from the method of Spear et al. (1989). An 80-μL sample of plasma was vortexed with an equal volume of ethanol to precipitate proteins. Retinol was dissociated from proteins by adding 80 μL of distilled water and extracted into 200 μL *n*-hexane with phase separation aided by centrifugation (15 000 × g; 2 min). A 160-μL aliquot of organic extract was evaporated to dryness at 45°C under a gentle stream of nitrogen gas. After cooling, retinol was redissolved in 60 μL ethanol for HPLC injection.

For retinyl palmitate (RP) and 3,4-dehydroretinyl palmitate (DRP) analysis in liver, intestine, testis, head kidney, and spleen, a method based on Spear and Moon (1986) was employed.

Because of the high concentration of lipids in ovaries, the analytical method of Boily et al. (1994) was used. This method provides high extraction efficiencies of retinoids from eggs while circumventing the need for elaborate clean-up procedures to remove excess lipids.

For tissues except ovaries, retinoids were analyzed using an HPLC system consisting of a model 590 pump, U6K injector, C-18 μ-Bondapak column (3.9 × 300 mm, 5 μm particle size), a model 441 absorbance detector set at 340 nm, and a chart recorder (all Waters Assoc., Milford, Mass.). Under the isocratic conditions used, the mobile phase of methanol (90% plus 10% water for ROH; 100% for RP and DRP) had a flow rate of 1 mL/min. Extracts of ovaries were analyzed on an HPLC system comprised of a model 126 pump, 210A injector equipped with a 100-μL loop, Ultrasphere ODS column (4.6 × 150 mm, 5-μm particle size), and a model 168 diode array detector (all Beckman Instruments, Montreal, Que.) controlled by an IBM model 55sx computer. The isocratic acetonitrile–chloroform (86.5:13.5) mobile phase flowed at 2 mL/min. The detector was set at 326 nm (RP) and 350 nm (DRP). Peaks were identified according to the retention times of authentic standards and confirmed by UV scans using the diode array detector. ROH and RP concentrations were quantified using external standards. For ovaries, quantification of DRP was based on values obtained from the RP standard curve multiplied by the correction factor 1.26 (the ratio of extinction coefficients for retinol, $E = 1832$; and 3,4-dehydroretinol, $E = 1450$). For liver and intestine, DRP was measured at 340 nm and quantified according to the correction factor of 1.1 (absorbance ratio of 3,4-dehydroretinyl palmitate relative to retinyl palmitate at 340 nm). Where retinoids could not be detected under the present HPLC conditions (the detection limit being approximately 2 ng), values of the limit of detection (i.e., 0.25 μg/g) were substituted. The means so estimated are therefore a conservative approximation.

Statistical analysis

The data were analyzed using the SAS software package for personal computers. Statistical comparisons were made by two-factorial analysis of variance (ANOVA) or covariance

Table 1. Condition and tolerance of adult brook trout (*Salvelinus fontinalis*) 8 weeks after a single i.p. injection of 5 μg/g BM 3,3′,4,4′-tetrachlorobiphenyl.

	Controls		3,3′,4,4′-Tetrachlorobiphenyl	
	Males (*N* = 16)	Females (*N* = 8)	Males (*N* = 9)	Females (*N* = 14)
CF (%)	1.45±0.17	1.45±0.25	1.38±0.10	1.38±0.15
HSI (%)	2.32±0.22	2.67±0.23	2.29±0.36	2.64±0.36
SSI (% × 10^{-1})	0.75±0.17	0.82±0.21	0.65±0.16	0.79±0.27
GSI (%)	0.63±0.54	0.82±0.28	0.63±0.52	0.61±0.13

Note: Values are means ± SD. CF, condition factor; HSI, hepatosomatic index; SSI, splenosomatic index; GSI, gonadosomatic index. The statistical significance of differences between groups was tested using one-way ANOVA with Duncan's test for specific comparison of means ($p \leq 0.05$).

(ANCOVA) using Duncan's multiple range test for specific comparison of means ($p \leq 0.05$). Data were log transformed to a normal distribution as necessary. Statistical testing of the ratio (RP:DRP) was only conducted when the distribution of each retinoid taken individually was normal.

Results

Brook trout

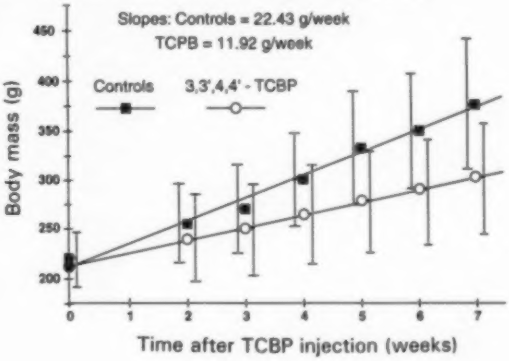
There were no significant differences between replicates; therefore, samples were pooled to form two groups: control and TCBP injected.

Growth curves were markedly different between the treatment groups (Fig. 1) although food consumption was normalized by pair feeding to an average of 1.5% BM/day. Slopes of the average body mass versus time were different ($p < 0.0001$) and the growth rate of controls was two times that of the TCBP injected group (slope values: 22.43 g/week controls vs 11.92 g/week TCBP-injected). Intercepts were not different ($p < 0.8092$) indicating that body masses were similar at the start of the experiment. Although the average feeding rate was 1.5% BM/day, a definite reduction in appetite was observed in the TCBP-injected group and this effect became progressively more pronounced with time. Food consumption decreased from 2% BM at the start of the experiment to 1% at the end. Consequently according to the pair-feeding regime, the diets given to controls were adjusted daily to the food consumption rate of the exposed group. The condition factors, HSI, SSI, and GSI did not differ significantly between TCBP-injected and control fish (CF, $p < 0.1773$; HSI, $p < 0.4632$; SSI, $p < 0.3867$; GSI, $p < 0.7666$) (Table 1).

In control brook trout, the quantity of DRP exceeded that of RP in both liver and ovary, while in intestines the quantity of RP was threefold that of DRP (Table 2). The possibility that unabsorbed food material (remaining in the intestine despite evacuation of intestinal contents) biased the analysis was rejected: retinoids were not detectable in trout chow extracted and analyzed by the same method used in the intestinal analyses.

Plasma retinol decreased in TCBP-injected brook trout ($p < 0.0037$) but was not different between sexes ($p < 0.0536$) (Table 2). Highly significant decreases in intestinal retinoid concentrations were revealed (RP, $p < 0.0143$; DRP, $p < 0.0009$) in TCBP-injected males, whereas in TCBP-injected females, retinoid levels did not decrease (RP, $p < 0.3223$; DRP, $p < 0.0597$). The concentrations of liver retinoids, when tested individually, did not vary between sex or treatment group. In contrast, the ratio of log RP to log DRP increased ($p < 0.0001$) in the TCBP-injected group. Logarithmic values were used in the ratio as these were normally distributed and the resulting ratio complied with the necessary conditions for parametric statistical analysis.

Fig. 1. Growth rate in brook trout (*Salvelinus fontinalis*) receiving a single i.p. injection of 5 μg 3,3′,4,4′-tetrachlorobiphenyl/g body mass. Values are means ± SD; *N* = 24 (controls); *N* = 23 (TCBP-injected fish).



No significant decrease in retinoids was apparent in ovaries (Table 2). Retinoids could not be detected in testis, spleen, or head kidney (pronephros) under the conditions of the present study (results not shown).

Lake sturgeon

Lake sturgeon from the reference site had detectable levels of retinoids, whereas most of fish caught in the contaminated Rivière des Prairies had undetectable retinoid levels (Table 3). In sturgeon from the reference site intestinal DRP concentration was twice that of RP (Table 3): RP and DRP contents of both females and males were significantly lower in sturgeon from the contaminated site as compared with those of the reference population (RP, $p < 0.0008$; DRP, $p < 0.0004$). RP concentration differed between sexes (RP, $p < 0.0002$), and there was a strong

Table 2. Retinoid concentrations in different tissues of brook trout (*Salvelinus fontinalis*) 8 weeks after a single i.p. injection of 5 μg/g BM 3,3′,4,4′-tetrachlorobiphenyl.

	Controls			3,3′,4,4′-Tetrachlorobiphenyl		
	Males ($N = 16$)	Females ($N = 8$)	Males + females	Males ($N = 9$)	Females ($N = 14$)	Males + females
Plasma						
ROH (ng/100 μL)	7.80 (5.94–9.66)	6.71 (5.28–8.14)	7.44 (5.67–9.21)	6.52 (4.94–8.10)	5.71 (4.36–7.06)	6.03*** (4.56–7.50)
Liver						
ROH (μg/100 g)	6.48 (2.00–10.96)	7.73 (3.82–11.64)	6.94 (2.73–11.15)	15.63* (2.95–28.31)	9.73 (2.14–17.32)	11.97 (2.01–21.95)
RP (μg/g)	12.53 (8.71–18.02)	10.82 (6.35–18.44)	11.93 (7.83–18.18)	15.68 (10.02–24.52)	13.58 (9.20–20.03)	14.36 (9.54–21.62)
DRP (μg/g)	14.83 (9.58–22.96)	13.69 (7.98–23.50)	14.44 (9.08–22.96)	12.30 (7.19–21.04)	10.58 (6.99–16.02)	11.22* (7.08–17.78)
RP/DRP	0.87 (0.74–1.04)	0.81 (0.67–0.98)	0.85 (0.71–1.02)	0.28 (1.07–1.54)	1.30 (1.01–1.67)	1.30*** (1.04–1.62)
Gonads						
RP (μg/g)	ND	10.25 (7.34–13.16)	—	ND	9.85 (5.56–14.15)	—
DRP (μg/g)	ND	11.84 (9.00–14.67)	—	ND	12.08 (7.32–16.85)	—
Intestines						
RP (μg/g)	13.36 (4.33–41.24)	5.04 (1.56–16.30)	9.65 (2.87–32.43)	3.12** (0.98–9.88)	3.27 (1.75–6.12)	3.22*** (1.40–7.36)
DRP (μg/g)	5.56 (2.21–13.99)	1.99 (0.66–5.99)	3.95 (1.34–11.65)	1.00*** (0.46–2.18)	1.02* (0.55–1.89)	1.01*** (0.52–1.96)
RP/DRP	2.65 (1.62–3.68)	2.58 (2.00–3.16)	2.63 (1.76–3.50)	3.29 (2.08–4.50)	3.43 (2.18–4.68)	3.38** (2.20–4.56)

Note: ROH, retinol; RP, retinyl palmitate; DRP, 3,4-dehydroretinyl palmitate. Values are means with ranges of SD given in parentheses. —, not determined; ND, not detected. Data for liver and intestine were log transformed for statistical analysis. The statistical significance of differences between groups was tested using one-way ANOVA with Duncan's test for specific comparison of means. *, $p < 0.1$; **, $p < 0.05$; ***, $p < 0.01$ (vs. controls).

interaction between sex and population ($p < 0.0210$). No effects as a result of sexual differences were apparent for DRP concentrations ($p < 0.2180$).

Discussion

The most important result of the present study is the profound decrease in intestinal retinoids of the TCBP-injected brook trout, which is consistent with the significantly lower intestinal retinoid concentrations in a contaminated lake sturgeon population. Chemical analyses conducted to date reveal that TCBP is the predominant coplanar PCB in the St. Lawrence sturgeon, reaching 1.1 μg/g in eggs, while the sum of coplanar PCBs was as high as 2.2 μg/g (P.A. Spear, A. Branchaud, and B. Bush, unpublished data), which are comparable to the dose level administered to brook trout in the present study.

The study demonstrates that visceral tissues, in this experiment an anterior segment of the intestine, have detectable levels of RP and DRP in brook trout (Table 2). Ganguly (1989) proposed that herring, salmon, and trout (genus and species not specified) may have more vitamin A in visceral tissues, particularly the pyloric caeca, than in the liver. The results indicate that the anterior segment of the intestine of brook trout is a storage site, but not a major storage site. The dramatic decrease in intestinal retinoids following TCBP injection might be considered as representing the loss of extrahepatic retinoids or the lack of ability to accumulate retinoids. Alternatively, this result may indicate a redistribution of retinoids between body organs as demonstrated previously in rats injected with coplanar PCBs or TCDD (Håkansson and Ahlborg 1985).

In the reference population of lake sturgeon, the DRP concentration was twice the RP concentration in intestines (Table 3); in contrast, the brook trout RP levels were threefold greater than DRP levels (Table 2). This finding may reflect interspecific differences in the type of retinoids stored in body tissues. For example, Goswami and Barua (1981) have classified freshwater fish from India as either retinol predominant or dehydroretinol predominant. Notwithstanding these interspecific differences, DRP decreased more rapidly than RP in brook trout, suggesting that DRP may be relatively sensitive to exposure to coplanar PCBs. One explanation for the apparently greater effect on DRP is preferential conversion by cytochrome P-450 or other induced enzymes involved in retinoid catabolism. Our results have demonstrated a significant increase of the hydroxylation of retinoic acid in trout liver microsomes

Table 3. Retinoid concentrations in intestine of lake sturgeons (*Acipencer fulvescens*) from a reference site on the Ottawa River and a contaminated site on the Des Prairies River near Montréal.

	Ottawa River			Des Prairies River		
	Males ($N = 5$)	Females ($N = 6$)	Males + females	Males ($N = 7$)	Females ($N = 2$)	Males + females
Intestines						
RP (µg/g)	0.52	2.48	1.22	0.26	0.25	0.26***
	(0.37–0.73)	(0.85–7.25)	(0.39–3.80)	(0.23–0.29)	(0.25–0.25)	(0.23–0.29)
DRP (µg/g)	4.79	4.52	4.64	0.43	0.25	0.39***
	(1.19–8.39)	(2.54–6.50)	(1.96–7.32)	(0.06–0.80)	(0.25–0.25)	(0.06–0.72)

Note: RP, retinyl palmitate; DRP, 3,4-dehydroretinyl palmitate. Values are means with ranges of SD given in parentheses. Data were log transformed for statistical analysis. The statistical significance of differences between groups was tested by two-factorial ANCOVA using Duncan's test for specific comparison of means. ***, $p < 0.01$ (vs. reference). Retinyl esters in six fish from Des Prairies River were below the limit of detection. Values of 0.25 µg/g (limit detection) were substituted and used for comparison with the reference site.

following injection of TCBP (Gilbert et al. 1995), although retinoid metabolism in the intestines has not yet been investigated.

The apparently greater effect on intestinal DRP is in agreement with the finding that 3,4-dehydroretinyl esters (DRE) undergo more rapid hydrolysis in the intestinal lumen than retinyl esters (RE) and are preferentially hydrolyzed and esterified within mucosal cells in a vitamin A depleted rat given an oral dose of both RE and DRE (Tanumihardjo and Olson 1988). The extra double bond in the ring of DRP, as compared with RP, may play an important role in these reactions. However, the activity of retinyl ester hydrolase during exposure to coplanar PCBs is uncertain. TCBP was found to be a noncompetitive inhibitor of retinyl palmitate hydrolase in rat (Powers et al. 1987; Mercier et al. 1990), while other researchers have reported the opposite effect. That is, acyl-CoA – retinol acyltransferase (ARAT) catalyzing retinol esterification was inhibited by coplanar biphenyls (Narbonne et al. 1990; Jensen et al. 1987). Again, the double bond in the ring of DRP may enhance its role in lipid peroxidation reactions associated with free radicals that might be generated by cytochrome P-450 induction.

A third possible explanation involves TCBP effects on stellate cells. Wake et al. (1986) have shown that in lamprey, hagfish, and eel (genus and species not specified), vitamin A storing stellate cells are found in the liver, intestine, kidney, ovary, testis, and gills. The fact that brook trout intestines contain detectable amounts of RP and DRP is itself consistent with the presence of intestinal stellate cells adjacent to enterocytes. Further studies are necessary to confirm the existence of intestinal stellate cells and their possible direct uptake of RE from mucosal cells, as well as the way RE are mobilized from stellate cells, following exposure of brook trout to TCBP. Mechanisms such as necrotic loss and transformation of stellate cells have been suggested by Chen et al. (1992) to explain PCB–PBBs and TCDD effects on the dynamics of vitamin A in the liver of rat.

The decrease in plasma retinol level may be attributed to metabolic disturbances, such as accelerated catabolism as a result of induced enzymes and inhibition of retinyl ester hydrolase. Interference of retinol binding to carrier proteins in the blood as occurs in mice injected with TCBP (Brouwer et al. 1988) may also have contributed to decreased blood retinol levels. However, another coplanar biphenyl did not interfere with the binding capacity of the carrier protein transthyretin (Spear et al. 1994). In rats injected with a coplanar biphenyl a decrease in plasma retinol was related to lower food consumption (Spear et al. 1994). Because of the fact that controls were pair fed, the lowering of plasma retinol in the present study cannot be strictly related to lower dietary intake.

A moderate effect on liver retinoids is indicated by the change in the RP:DRP ratio in liver of the brook trout (Table 2). As in the case of intestinal retinoids, these results suggest that DRP is more sensitive than RP to the effects of TCBP and are consistent with our field data showing decreased liver retinoids in contaminated areas (Spear et al. 1992). Palace and Brown (1994) have observed decreased liver retinol, dehydroretinol, and retinyl palmitate concentrations in lake char, *Salvelinus namaycush*, exposed to orally administered coplanar 3,3′,4,4′,5-pentachlorobiphenyl. The decrease in liver retinoid levels in laboratory mammals following exposure to cytochrome P-450-inducing contaminants is well known (Villeneuve et al. 1971; Chen et al. 1992; Azais et al. 1987; Brouwer and Van Den Berg 1984), and accelerated retinoid metabolism has been demonstrated in liver microsomes following the injection of a coplanar biphenyl (Spear et al. 1988).

However, a clear effect on an individual retinoid in liver was not demonstrated in the present study. Interspecific differences either in response to TCBP or in retinoid metabolism may be associated with the lack of a pronounced effect on liver retinoid concentrations. Also, the 2-month exposure via a single injection of TCBP may not have been sufficient to elicit the same degree of effect as chronic multiple exposures in contaminated environments. For instance, Spear et al. (1989) found no change in liver retinyl palmitate in ring doves, *Streptopelia risoria*, injected twice with 40 µg TCBP/g, while liver retinol was significantly lower in the TCBP-injected doves. In contrast, herring gulls, *Larus argentatus*, nesting at a contaminated site on Lake Ontario had significantly lower hepatic levels of both retinol and retinyl palmitate compared with birds from less-contaminated areas (Spear et al. 1986). An in-field experiment demonstrated that injection of 2,3,4,7,8-pentachlorodibenzofuran (P_5CDF) resulted in a decrease in liver retinoids in male lake trout only after a chronic exposure (Delorme et al. 1993). Alternatively, the minor effect of TCBP on liver retinoid stores in the present study may have been related to the pair-feeding experimental design. Food consumption by the control trout would have been substantially greater than that of the TCBP group had the controls not been pair fed. This means that the vitamin A dietary intake in control animals would have been greater under ad libitum feeding and would have contributed to the difference in liver retinoid stores between control and TCBP groups.

Marked growth effects were found in the present study despite the pair-feeding design. We consider that the daily adjustment of diet normalized the TCBP-related loss of appetite. Thus, the growth reduction was independent of food intake and is considered to be a physiological bioenergetic effect rather than a behavioural (i.e., feeding) effect of the toxicant. Such growth effects may have profound implications for fisheries productivity and management strategies.

An unexpected finding was that the condition factor, HSI, SSI, and GSI appeared to be similar in both the TCBP-injected and control groups (Table 1). Hepatomegaly, or increased HSI, is a typical effect of PCBs in laboratory mammals. As well, growth effects in mammals exposed to PCBs, dioxins, and related compounds are characterized by the progressive loss of mass, "wasting syndrome" (or lack of growth in juveniles), which in fish should be indicated by a decrease in the condition factor. It is curious, therefore, that TCBP apparently did not elicit a wasting syndrome - type effect in the present study.

The appetite loss was progressive throughout the experiment. That is, the TCBP-injected fish were initially eating approximately 2% BM/day but their food consumption rate decreased to 1% 8 weeks later. We further observed that the appetite loss appeared to be greater at lower ambient temperatures than at higher temperatures within the 9–13°C range of this study. Stalling et al. (1979) reported decreased food consumption, growth reduction, and disorientation in rainbow trout exposed to TCBP in the water leading to tissue residues ranging from 0.8 to 12.0 μg/g of the biphenyl.

Sufficient dietary vitamin A is required for normal physiology of fish, and deficiency has so far been shown to cause growth retardation, edema, and corneal lesions (Poston et al. 1977). The symptoms of dioxin exposure in humans and laboratory mammals has been likened to the symptoms of vitamin A deficiency (Vos 1978). Chick edema disease is caused by such contaminants in wild and captive birds and a similar syndrome exists in fish (Gilbertson et al. 1991; Walker et al. 1991). Growth effects and retinoid imbalances reported in the present study are congruent with these previous findings.

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Dynamic modelling of phycotoxin kinetics in the blue mussel, *Mytilus edulis*, with implications for other marine invertebrates

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Abstract: Modelling the uptake and detoxification kinetics of phycotoxins in marine filter-feeding bivalve molluscs is discussed and illustrated by a case study involving blue mussels (*Mytilus edulis*) in the lower St. Lawrence estuary in eastern Canada. A dynamic model was fit to empirical data acquired on differential responses of mussels transplanted from sites characterized by differing history of exposure to toxigenic blooms responsible for paralytic shellfish poisoning (PSP). Although it is difficult to collect sufficient data to calibrate complicated models, it appears that one- and two-compartment models are fully adequate for this type of modelling. Measuring phycotoxin levels in shellfish can be a useful and cost-effective adjunct to direct monitoring of phytoplankton toxicity in the water column, because ongoing filtration by shellfish provides an integrated estimate of the toxin to which they are exposed.

Résumé : Nous examinons la modélisation de la cynétique de l'absorption et de l'élimination des phycotoxines chez des mollusques bivalves filtreurs, et nous l'illustrons par une étude de cas portant sur la moule bleue (*Mytilus edulis*) du bas estuaire du Saint-Laurent, dans l'est du Canada. Un modèle dynamique a été ajusté aux données empiriques recueillies sur différentes réactions de moules provenant de sites caractérisés par des histoires différentes d'exposition aux efflorescences toxiques responsables de l'empoisonnement par la toxine paralytique (PSP). Bien qu'il soit difficile de recueillir suffisamment de données pour étalonner des modèles compliqués, il semble que les modèles à un et à deux compartiments sont parfaitement satisfaisants pour ce type de modélisation. La mesure des concentrations de phycotoxines dans les coquillages peut constituer un moyen utile et rentable pour compléter la surveillance directe de la toxicité du phytoplancton dans la colonne d'eau, car la filtration en continu de l'eau par les coquillages permet d'obtenir une estimation intégrée de la quantité de toxine à laquelle ils sont exposés.

[Traduit par la Rédaction]

Introduction

Accumulation of phycotoxins in marine invertebrates, including molluscs and crustaceans, is a major public health concern in coastal zones throughout the world (Shumway 1990). In regions where toxigenic blooms are frequent events, such as in northern Europe, Japan, and the Pacific and Atlantic coasts of North America, both recreational and commercial exploitation of shellfish resources is severely restricted (Shumway and Cembella 1993). Shellfish vary widely in their capacity to ingest toxigenic microalgae and to sequester phycotoxins in various anatomical compartments (Lassus et al. 1989; Bricelj et al. 1990, 1991; Bricelj and Cembella 1995). Bivalve molluscs typically acquire phycotoxins through direct filter-feeding upon toxic microalgae from pelagic and benthic sources (Shumway et al. 1987), whereas accumulation in crustaceans and gastropods is most often the result of secondary transfer through the food chain (Shumway 1995).

Among the major phycotoxin syndromes, paralytic shellfish poisoning (PSP) has the longest recorded history and has accounted for the majority of human fatalities. As a consequence, many countries have implemented toxin monitoring strategies yielding several decades of comprehensive PSP toxicity data from a variety of species collected from fixed stations (Quayle 1969; Prakash et al. 1971; Shumway et al. 1988). More recently, toxicity data has been supplemented by routine monitoring of toxic phytoplankton blooms in an effort to acquire baseline time-series data on bloom dynamics and to provide an early warning of impending deleterious environmental effects (ICES 1992). Experiments on toxin kinetics in controlled laboratory systems that have utilized sophisticated analytical methods

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to determine toxin content and composition (Lassus et al. 1989; Bricelj et al. 1990, 1991; Bricelj and Cembella 1995) have provided detailed insights into uptake mechanisms and toxin kinetics for a rapidly growing list of shellfish species. Seasonal data on toxin compositional changes in field populations are also available for a few species (Martin et al. 1990; Oshima et al. 1990; Cembella et al. 1993, 1994).

Determining the kinetics of phycotoxin uptake and detoxification in marine invertebrates is essential to understanding the fate of these toxins in marine food webs and also has important implications for toxic algal bloom dynamics in coastal waters. In spite of limited attempts to describe seasonal and geographical patterns in shellfish toxicity (Gaines and Taylor 1985; Shumway et al. 1988; Cembella et al. 1994), to examine long-term cyclical trends in toxicity (White 1982), and to develop toxicity indices for monitoring strategies (Chiang 1988), dynamic models for phycotoxin kinetics are not widely used. This is surprising, because even very simple models with only one or two compartments can provide useful insights on both the presence of toxins in the water column and on their distribution in shellfish (Silvert and Subba Rao 1992; Lee 1993). Even these simple models suggest that toxin analysis of shellfish tissues may be a valuable adjunct to routine monitoring of toxic blooms, and for assessing and hindcasting the abundance and cellular toxicity of microalgal populations. Particularly desirable are models that predict (*i*) the maximal uptake rate and body burden of toxin, (*ii*) differences in the seasonal persistence of toxicity in various species, (*iii*) the anatomical distribution of toxins, and (*iv*) progressive shifts in toxin composition indicative of biotransformation or selective retention.

The present modelling exercise arose from a comparative study of the kinetics of the phycotoxins responsible for PSP in different populations of the blue mussel (*Mytilus edulis*) (Chebib et al. 1993). The objective of this earlier study was to determine whether toxin uptake and detoxification rates in mussels transplanted to a site where they were exposed to natural blooms of PSP toxin-producing dinoflagellates depend upon previous exposure to these phycotoxins. The experimental results showed that mussels which had a history of chronic exposure to PSP toxins accumulated more toxin and achieved a higher maximum toxicity during a toxic bloom than mussels which had not been previously exposed. The objective of the dynamic modelling presented here was to interpret the uptake and detoxification kinetics of PSP toxins in a representative filter-feeding bivalve mollusc.

The blue mussel (*Mytilus edulis*) as a test species

The blue mussel (*Mytilus edulis*) was selected as the candidate species for phycotoxin dynamic modelling because its feeding responses, growth kinetics, ecology, and physiology have been well described and quantified in the general literature (see Gosling 1992 for comprehensive reviews). In particular, the detailed response of mussels to varying cell densities and specific PSP toxicities has been well characterized under controlled conditions in the laboratory (Bricelj et al. 1990; Lee 1993). Furthermore, a complete time-series seasonal data set on PSP toxin accumulation and water column toxicity was available (Chebib 1992; Chebib et al. 1993). Among bivalve molluscs, mussels are known to accumulate phycotoxins rapidly, but they also detoxify completely within several weeks under field conditions (Shumway et al. 1988; Chebib et al. 1993). In contrast, the surfclam *Spisula solidissima* is known for prolonged PSP toxin retention (>1 year) (Shumway et al. 1994). Within a hierarchy of bivalve molluscs, mussels are considered among the most resistant to the neurophysiological effects of purified PSP toxin (saxitoxin) on isolated nerves (Twarog and Yamaguchi 1975). As one possible consequence, mussels can sustain high filtration rates when exposed to unialgal cultures of PSP-toxin producing dinoflagellates (Shumway and Cucci 1987; Bricelj et al. 1990) and can tolerate a total toxin body burden greater than 45 000 μgSTXeq (saxitoxin equivalents) per 100 g of soft tissues (560 times the accepted regulatory limit for human consumption) with little evidence of physiological impairment (Bricelj et al. 1990). In fact, mussels grow well when fed upon a monospecific diet of a dinoflagellate which produces PSP toxins, albeit with a lower condition index than with a non-toxic food source (Bricelj et al. 1993). Some apparently contradictory evidence has suggested that mussels with no prior history of exposure to PSP toxins may suffer high mortalities and physiological signs of stress when challenged with toxigenic cells (Shumway and Cucci 1987). Because of their capacity for rapid uptake and retention of marine toxins, and their widespread distribution in coastal ecosystems, mussels have been selected as a sentinel organism for monitoring PSP toxicity (Shumway et al. 1988), as well as for a variety of other marine environmental contaminants in "Mussel Watch" programs (Widdows and Donkin 1992).

In the present study we developed a dynamic model of phycotoxins using data collected from two natural populations of *Mytilus edulis* transplanted to a common site in the lower St. Lawrence estuary in eastern Canada (Chebib et al. 1993). Blooms of the toxic dinoflagellate *Alexandrium excavatum* are a recurrent cause of toxicity associated with PSP in the lower St. Lawrence estuary and general bloom dynamics have been described in this region (Therriault et al. 1985; Cembella and Therriault 1989). Typical abundance of toxic *Alexandrium* cells during blooms in the region may approach 10^5 cells·L^{-1}, but concentrations exceeding 10^6 cells·L^{-1} have been observed in stratified layers associated with river plumes (Therriault et al. 1985). In addition, because the cellular toxicity of natural *Alexandrium* populations in the lower estuary is among the highest ever recorded (Cembella and Therriault 1989; Chebib et al. 1993), toxin levels in shoreline molluscan bivalves rise rapidly during blooms to levels greater than 20 000 μgSTXeq (100 g)$^{-1}$.

Time-series data were collected on the seasonal variation in concentration of toxigenic dinoflagellates at the experimental mussel site in the lower estuary, as well as on toxin concentrations in the water column and in excised mussel digestive glands (Chebib et al. 1993). Toxin content and composition in mussel digestive glands ($n = 4$) were determined from specimens collected simultaneously with phytoplankton samples (sometimes containing toxigenic dinoflagellate cells) from the same fixed depth in the water column (3 m) throughout two successive bloom periods. Toxin measurements based upon vertical net tow samples from the water column component were available on only a few dates when toxic cell numbers were sufficient for accurate analysis (more than 1000 cells·L^{-1}).

Supplementary empirical data on mean filtration rates were obtained for the modelling exercise by feeding replicate individual mussels (n = 8) under simulated natural conditions in the laboratory (Chebib et al., in preparation). Natural seston assemblages were supplemented with cultured *Alexandrium* cells produced from a clonal isolate from the St. Lawrence estuary, to yield a concentration of toxic cells similar to that experienced by the mussels during the early summer bloom. Since clearance rates of mussels are a function of such factors as size, soft tissue weight, age, and reproductive status, care was taken in the selection of comparable specimens for the field transplant and filtration rate experiments.

One of the critical questions in interpreting phycotoxin dynamics is whether shellfish can acclimate to frequent blooms, and whether any such response arises through avoidance (reduced filtration) or by other altered physiological mechanisms such as increased toxicity tolerance or enhanced detoxification rates. We have compared the uptake and detoxification of mussels with different histories of exposure to PSP toxins in an effort to address this issue. The model simulations indicated apparent discrepancies between the measured toxin concentrations in the water column and in mussels, which cannot be explained by refinements in modelling.

Model development

General models of phycotoxin kinetics

The kinetics of phycotoxins within shellfish involve a balance between several processes: (*i*) ingestion of toxic algae, (*ii*) digestion and assimilation efficiency, (*iii*) transfer of toxins among organs, (*iv*) biotransformation into other toxic derivatives, (*v*) detoxification through various loss processes. A complete model of phycotoxin kinetics therefore involves describing and quantifying all these processes.

For suspension-feeding invertebrates, including bivalve molluscs, the primary (and perhaps exclusive) route of phycotoxin entry is via ingestion of toxic cells. Certain species are capable of bioaccumulation of dissolved organic contaminants from seawater against huge concentration gradients, with partitioning into tissues usually behaving as a direct function of increasing hydrophobicity (Widdows and Donkin 1992). In the case of highly aqueous-soluble phycotoxins, such as PSP toxins and domoic acid, which are not excreted to a significant extent by healthy phytoplankton, little direct absorption from seawater or passage through the exposed ctenidia (gills) or mantles would be expected. Evidence shows that, regardless of species-specific differences in digestive physiology and toxin assimilation efficiency, the digestive system is the main initial repository of phycotoxins in filter-feeding bivalves after exposure to natural toxic blooms (Shumway and Cembella 1993; Cembella et al. 1993) and when toxic algal cells are fed to bivalves in controlled laboratory experiments (Bricelj et al. 1990; 1991).

The clearance rate (volume swept clear per unit time, CR) of a suspension-feeding invertebrate can be determined as

$$[1] \quad \mathrm{CR} = V(\ln C_i - \ln C_f)/(t_f - t_i) = -V\,\Delta \ln C/\Delta t$$

where C_i and C_f are the initial and final particle concentrations and V is the volume of the container used in the experiment (Coughlan 1969). The clearance rate for bivalve molluscs is known to be an allometric function of the body weight of soft tissues (reviewed extensively by Bayne and Newell 1983). The ingestion rate (I) is obtained by multiplying the clearance rate by a correction factor ϵ which represents a combination of filtration efficiency and cell loss because of pseudofaeces production, and multiplying this by the instantaneous concentration of particles retained with 100% efficiency (Foster-Smith 1975). As is the general case for suspension-feeding bivalves, filtration efficiency for mussels is extremely high, ranging from 80 to 100% for particles as small as 2 μm (Møhlenberg and Riisgård 1977), so the value of ϵ is probably close to unity. For the modelling exercise, we assumed a value of $\epsilon = 1$, because no pseudofaeces production was observed in the clearance rate determinations and seston loads were below the typical threshold for selection. Mussels exhibit considerable variability in pre- and post-ingestive particle selection, involving preferential retention on the gills, rejection through pseudofaeces production, and differential post-ingestive processing in the digestive system (Hawkins and Bayne 1992).

Growth rate is governed mainly by the nutrient supply and uptake rate and absorption efficiency, which is dependent upon the food quality of the ingested phytoplankton and detritus (Thompson 1984; Page and Hubbard 1987). Physiological energetic studies on mussels have demonstrated that there is little effect of water temperature on scope for growth in the range from 10 to 20°C (Bayne et al. 1976) and mussels acclimate rapidly to optimize filtration rates in response to temperature fluctuations. In the St. Lawrence estuary study (Chebib et al. 1992), the water temperature at 3 m depth varied seasonally from 5 to 12°C from May to the end of October, but remained nearly constant at 10°C during and subsequent to the two summer bloom periods. Thus, a Q_{10} temperature effect need not be incorporated into this dynamic model.

One of the most difficult problems in modelling toxin kinetics is that many toxigenic microalgae produce a suite of chemically related phycotoxins rather than a single toxic compound. This is particularly true for the PSP toxins: more than 20 derivatives of saxitoxin (STX) have been identified among various toxic dinoflagellates and within the tissues of marine invertebrates (Cembella et al. 1987; 1993; Oshima et al. 1990). These compounds differ greatly in specific potency (ranging over almost two orders of magnitude) and net toxicity may be enhanced through facile biotransformation processes occurring within shellfish tissues. This is contrary to the usual effect of catabolic digestive processes in bivalve molluscs that typically lead to a substantial reduction in net toxicity of accumulated xenobiotics (Widdows and Donkin 1992).

Via metabolic transformations, certain shellfish are capable of profoundly modifying the composition of ingested toxins (Sullivan et al. 1983; Cembella et al. 1993). The kinetics of these biotransformation processes for PSP toxins in natural populations are highly species-specific, whereas they are much less subject to geographical variation among populations (Cembella et al. 1993; 1994). There is no evidence to date that bivalve shellfish are capable of completely degrading PSP toxins to nontoxic components.

Domoic acid, a potent neurotoxin associated with amnesic shellfish poisoning (ASP), along with relatively small quantities of its isomers has been identified in toxigenic phytoplankton and in shellfish (Quilliam et al. 1989). However, the toxicity of these domoic acid analogues and their susceptibility to biotransformation in shellfish has yet to be determined. Similarly, for the diarrhetic shellfish toxin complex (DSP toxins) (Yasumoto 1987), which includes the structurally related compounds okadaic acid, dinophysistoxins, and several recently identified diolester derivatives (Hu et al. 1993) (plus pectenotoxins and yessotoxins found exclusively in shellfish), dynamic modelling of biotransformation processes awaits further work on the relative biological activity of these compounds.

It is conceivable that differential binding and chemical stability of related phycotoxins could yield asymmetric rates of uptake, assimilation and detoxification, resulting in a shift in toxin composition and net toxicity. This has not yet been demonstrated in laboratory simulations because of the lack of sufficient purified toxin analogues. Fortunately the processes involved in toxin kinetics appear to involve only first-order processes, so standard methods of compartmental modelling can be used. Therefore, we can assume that in modelling the rate of biotransformation from one toxin into another, $A \rightarrow B$, the rate is $b \times A$ where b is the biotransformation rate and A is the concentration of A. Similar assumptions can be made about the transfer of toxins from one compartment to another. Thus, a general equation for the concentration C_{si} of toxin s in compartment i (which generally refers to a group of tissues) can be written in the form

$$[2] \quad \mathrm{d}C_{si}/\mathrm{d}t = \Sigma_{s'}(b_{s'si}C_{s'i} - b_{ss'i}C_{si}) + \Sigma_j\,(k_{sji}C_{sj} - k_{sij}C_{si}) - e_{si}C_{si}$$

where $b_{ss'i}$ is the biotransformation rate of toxin s into toxin s' in compartment i, k_{sij} is the rate at which toxin s is transferred from compartment i to compartment j, and e_{si} is the detoxification (loss) rate for toxin s from compartment i. The first combined term, $(b_{s'si}C_{s'i} - b_{ss'i}C_{si})$, represents the balance between biotransformation of other toxin concentrations $C_{s'i}$ into C_{si} and the inverse reaction, and second term $(k_{sji}C_{sj} - k_{sij}C_{si})$ represents the physical transfer of toxin into and out of compartment i, and the final term $e_{si}C_{si}$ includes other loss processes such as defecation and excretion.

Simplification of the model

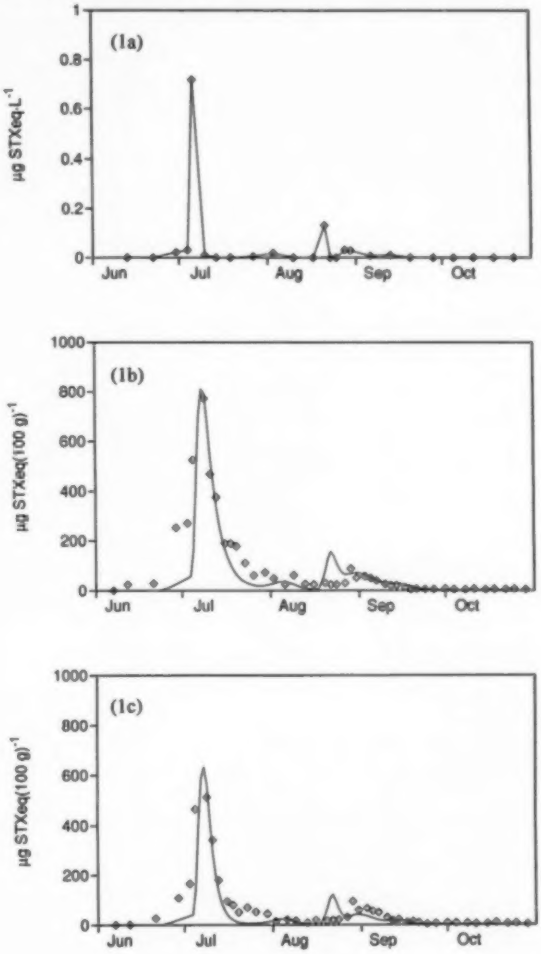
Although a general model of phycotoxin kinetics provides a useful starting point, there are very limited biological field data that can be used to estimate enough parameters to construct any but a very simple model. Recent studies of two species of bivalve molluscs under controlled conditions in the laboratory (Lee 1993) suggest that a two-compartment model based upon the uptake and detoxification kinetics of PSP toxins might be sufficient to describe toxin dynamics of whole shellfish. In Lee's (1993) analysis these two compartments were defined as the viscera (digestive gland and associated tissues), where phycotoxins first enter the organism, and other tissues which receive toxins via transfer from the viscera. Of these, the viscera exhibits much faster rates, and detoxification generally proceeds in two stages: (*i*) rapid loss of toxin (primarily in the faeces) because of clearance of unassimilated toxin from undigested cells or in dissolved form in the gut lumen, and (*ii*) slower release of assimilated toxin either bound to the viscera or incorporated within other tissues. Detoxification of the other tissues can occur either by transfer of toxins back to the viscera from where they are ultimately excreted, or perhaps by direct loss through leakage or diffusive excretion from the surface of these organs, as has been hypothesized for PSP toxin loss from the mantles of Japanese scallops (Inoguchi et al. 1990).

In the present study, only the toxin load in the mussel viscera was routinely measured, so toxin present in other tissues could not be used to define an additional compartment. Nevertheless, weekly toxin analysis performed independently on the viscera and other tissues towards the end of the experiment during October, yielded PSP toxin levels in these other tissues that were much lower than in the viscera, generally less than 5% of the total body burden. This low proportion of toxins in the nonvisceral tissues was also representative of that found for mussels in controlled feeding experiments (Bricelj et al. 1990), even at the peak of toxin uptake; therefore, exclusion of the other tissues from the model does not seriously compromise its validity. In our two-compartment model of detoxification in mussel viscera, the two functional toxin compartments designated as "labile" and "bound" may correspond operationally to those defined by Lee (1993), if we interpret the labile fraction as unassimilated toxin free in the gut or retained within undigested dinoflagellate cells, and the bound fraction as toxin attached to the gut wall or incorporated within the tissue. However, the exact anatomical location of these functional toxin compartments and the mechanisms by which toxin binding occurs remain to be ascertained.

Biotransformation is extremely important in interpreting phycotoxin dynamics, but little information is available regarding in vitro conversion rates in the laboratory, and even less is known from field populations of invertebrates. This subject was reviewed recently for two bivalve species from the Gulf of Maine (Cembella et al. 1993), but unfortunately all data on toxin compositional changes were derived from discrete point-source specimens sampled weekly or monthly over a long time series (2 years), revealing little about the rate constants required for kinetic modelling of toxin biotransformation. Nevertheless, it was clear that biotransformation occurs on a shorter time scale (hours to days) than the sampling frequency of most toxic shellfish monitoring programs (weeks to months).

Biotransformation affects toxin kinetics in two crucial ways: first, net toxicity can change, even increasing in the absence of new input of exogenous toxin, and second, the rates at which catabolized toxins are transferred between organs or excreted may vary widely. For this modelling exercise, we have chosen to overlook the role of biotransformation and to treat the kinetics of all toxins as the same. This is equivalent to considering only one toxin analogue, and although this is seldom the case, it is often a necessary initial step because of limited information. Compared with certain other molluscan bivalve species, particularly the little-neck clam *Protothaca staminea* (Sullivan 1983) and the surfclam *Spisula solidissima* (Cembella et al. 1993), PSP toxin composition in mussels is subject to much less biotransformation under both field conditions (Chebib et al. 1993) and in controlled laboratory feeding experiments (Bricelj et al. 1990). In field populations, the degree of epimerization and conversion of low potency *N*-sulfocarbamoyl toxins to more toxic carbamate derivatives did not yield dramatic increases in net toxicity subsequent to exposure to a toxic bloom (Chebib et al. 1993). Hence, at least for mussels, it is a reasonable approximation to model the dynamics of net toxicity without invoking biotransformation kinetics among compartments, because net toxicity generally tracks the total toxin concentration.

Fig. 1. Comparison of simulation results for a one-compartment model to field data on mussel toxicity from the lower St. Lawrence estuary. The water column toxicity values obtained by multiplying *Alexandrium* cell density by toxicity per cell are shown in *a*. Simulated mussel toxicity (solid lines) is plotted against data (points) for specimens transferred from Cap Chat (*b*) and the Magdalen Islands (*c*).



With this set of simplifying assumptions, we can write a basic two-compartment and one-toxin model of the passage of phycotoxins through the following equations:

[3] $dC_0/dt = I - k_{01}C_0 + k_{10}C_1 - e_0C_0$

[4] $dC_1/dt = + k_{01}C_0 - k_{10}C_1 - e_1C_1$

where C_0 is the concentration of labile toxin in the viscera, C_1 is the concentration of bound toxin, I is the ingestion rate, and the k_{ij} and e_i coefficients (where i and j represent the indices 0 and 1) are the transfer and detoxification rates as described earlier.

We can simplify this model further if we assume that k_{01} and k_{10} are small enough to be neglected, in which case we are left with the one-compartment model

[5] $dC/dt = I - eC$

where C_1 is zero so the subscript 0 on C_0 can be omitted. Although this may seem an extreme degree of simplification, it is often the only appropriate model to use. Phycotoxin determinations in shellfish tissues are usually based either on the viscera only or on homogenates of whole animals; adequate analyses of the viscera separated from the other tissues are rarely obtained. Use of the viscera alone for modelling detoxification rates has the advantage of minimizing the effect of toxin "dilution" through allometric growth of individual tissue compartments over long time-series (months to years). However, even when separate measurements are not available, the data sometimes suggest the existence of a second compartment. For example, Silvert and Subba Rao (1992) observed that the high levels of domoic acid found in mussels at certain times could be explained by retention of the toxin in tissues with slower detoxification rates than the viscera. In this context, it is important to recognize that deviations from a model can be as informative as the output of the model itself.

Modelling toxic phytoplankton dynamics

Although we have focussed on modelling the uptake and detoxification kinetics of shellfish, the toxic cell density and water column toxicity that drive the model are continuous variables, whereas the data are discrete. Deriving a continuous function from discrete data involves assumptions about how the data vary between sampling dates, which in turn implies the use of a model. Use of an inapplicable model can lead to serious interpolation errors, known as *aliasing* problems.

If data vary slowly and in a regular fashion, one can use a range of interpolation schemes such as linear interpolation, exponential smoothing, or splines. For the experimental data set (Chebib 1992) used for the modelling simulations, most of these smoothing techniques are inapplicable. For example, the *Alexandrium* cell counts at 3 m depth were zero on August 9, 16, 22, and 24, but 10 000 cells·L^{-1} were recorded on August 20. Because high cell numbers were also present at the surface and at 7 m depth on the latter date, this does not represent a spatial sampling artifact on the vertical scale. Either there was a brief bloom between August 16 and 22, or a transient patch of dinoflagellates may have passed through the system on August 20 which was rapidly removed by grazing or advection. In the first case, the *Alexandrium* cell count on August 18 might have also been 10 000 cells·L^{-1} (or even higher), whereas in the second case it might have been zero.

We have chosen to use linear interpolation to estimate the water column toxicity, as shown in Fig. 1*a*. Exponential smoothing cannot be used with data containing zeroes, and splines would generate negative values. A more sophisticated model based on known patterns of phytoplankton patchiness could perhaps be used as the basis for a series of Monte Carlo simulations, but that goes far beyond the scope of the present work.

Simulations

Parameter values

Parameter estimation and sensitivity analysis are essential parts of modelling (Silvert 1981), but the data available for this study were not sufficient for the estimation of more than one or two parameters. A fixed filtration rate of 6.4 L·d^{-1}·g^{-1} (wet weight of soft tissues) was assumed for all simulations for both transplanted mussel populations, based upon the results of the clearance rate experiments performed with supplemented natural phytoplankton assemblages from the St. Lawrence estuary (Chebib et al., in preparation), and from previous mussel feeding experiments with unialgal cultures of *Alexandrium* (Bricelj et al. 1990). Although filtration rate is known to vary seasonally and depends upon seston load and quality, the use of a constant value is sufficient for the type of models used here. Other parameter values were estimated by fitting the data. For the one-compartment model, only one other parameter was needed, the detoxification rate, and the results of the simulation did not appear very sensitive to this parameter. However, the fitted detoxification rate for pristine mussels from the Magdalen Islands (MAD) is almost twice as high (40 vs. 24%·d^{-1}) as that for mussels from Cap Chat (CAP). This suggests that mussels which are chronically exposed to PSP toxins (CAP) are adapted physiologically and do not purge the toxins as rapidly as mussels without this history. To test this hypothesis it would be best to develop a model that incorporates aspects of toxin exposure history and for which the detoxification time varied in response to the quantity of phycotoxins present, but the present data set was insufficient to test and validate such a model. Similarly, we obtained better results with a two-compartment model, but could not test this model fully with the data available.

Simulation results

Numerous simulations were carried out, three of which are discussed here. The fit is quite good using the one-compartment model described by [5] as shown in Fig. 1, but there are three major discrepancies between the simulation results and the data which need to be addressed. These are (1) the simulation shows a level of toxicity in both the CAP and MAD mussels which is too low at the beginning of the primary bloom, (2) there is a simulated spike in mussel toxicity just before the secondary bloom that is not reflected in the data, and (3) at the end of the primary bloom the simulated toxicity falls off more rapidly than was measured.

Each of these discrepancies can be attributed to one or more of the following: (*a*) errors in measurement of toxicity in mussels; (*b*) errors in the values used for toxicity in the water column. This can be due to inaccurate cell counts or to inaccuracies in determining the toxicity per cell. The latter parameter is subject to a compound error because it is the product of the number of cells (precision of counts >90%) and the analytical error inherent in the toxin determination (precision >97%). Vertical migration of toxic cells and temporal patchiness also lead to unrepresentative sampling, which is expected to be more significant than analytical error. Aliasing errors in the values for water column toxicity can arise if an inappropriate model of algal density is used to convert point data into continuous functions; (*c*) errors and omissions in the model. In our analysis we focus on items (*b*) and (*c*), since measurement of toxicity in mussels and algal cells is straightforward and does not appear to be subject to serious errors. Analytical precision in determining toxin concentration is >97% for replicate injections ($n = 3$), whereas for pooled mussel samples ($n = 4$), individual variation among samples is typically less than 10%.

Problem (1) presents a very clear picture. Because we detect substantial levels of toxin in mussels before there is any measurable level of toxicity in the water column (Fig. 1*a*), any model which assumes that the toxicity in mussels comes exclusively from the water column cannot be expected to predict or explain the measured values. Note that the *Alexandrium* cell counts used for determining water column toxicity are from the same depth as the mussel cages (3 m). This problem cannot be resolved by adding compartments or otherwise refining the model. We must conclude either that the toxicity in the mussels does not come from the water column and/or that there is an ingestible source of PSP toxin other than vegetative *Alexandrium* cells, which is unlikely, or that the data on cell-specific dinoflagellate toxicity in the water column are the source of error. The large mesh size (>1 cm) of the mussel cages and the relatively low stocking density essentially precluded nonhomogeneous distribution of filterable phytoplankton or the creation of unrepresentative microdepletion zones in this highly dynamic environment (residual current velocity >20 cm·s^{-1}). The potential contribution of benthic *Alexandrium* cysts was negligible, as no cysts were ever recovered from the vertical net tows during the experiment, and historical distribution maps indicate the presence of only very low numbers of toxigenic cysts at this site (Turgeon et al. 1990).

An alternative suggestion is that the toxin in the mussels may continue to be synthesized by *Alexandrium* cells that are still biologically active in the shellfish gut (J.W. Sowles and D.J. Silvert, personal communication). The contribution to internal toxin pools through in situ cell growth and toxin production could be substantial, given reasonable gut residence times for *Alexandrium* cells which can be more than a day (A. Cembella and H. Chebib, unpublished observations). However, quantitative data are insufficient to test this hypothesis.

Problem (2) presents the opposite pattern: a measured peak in *Alexandrium* cell concentration gives rise to a simulated peak in mussel toxicity which is not seen in the data. The situation is not as clear as for problem (1), since a hypothesis to consider is that the mussels could respond to high toxin levels in the water column by reducing their filtering rates, thereby decreasing ingestion of toxic cells. However, evidence from laboratory feeding studies on mussels (Bricelj et al. 1990) suggests that *Alexandrium* cell densities of 10 000 cells·L^{-1} are well below the threshold that would cause a reduction in filtration rate resulting from toxin loading. This is particularly true since the range of toxicities found in field populations of *Alexandrium* from the St. Lawrence estuary (3.3 to 13.8 pg STXeq·cell^{-1}) was below that of the higher toxicity isolates fed to mussels in the laboratory (Lee 1993). Given the three-dimensional patchiness of phytoplankton, we suspect that this discrepancy arises from a transient pulse of toxic dinoflagellates, and certainly the discontinuous peak shown in Fig. 1*a* is suspicious and perhaps indicative of a discrete sampling artifact. As pointed out previously, by using linear interpolation to model water column toxicity between sampling dates we can transform correct data into incorrect average concentration estimates. For example, if the high concentration measured on August 20 was due to a small but concentrated patch of *Alexandrium* advected through the study site at a depth of 3 m, the peak would be much narrower than that shown in Fig. 1*a* and the integrated potential amount of toxin ingested by mussels would be greatly reduced.

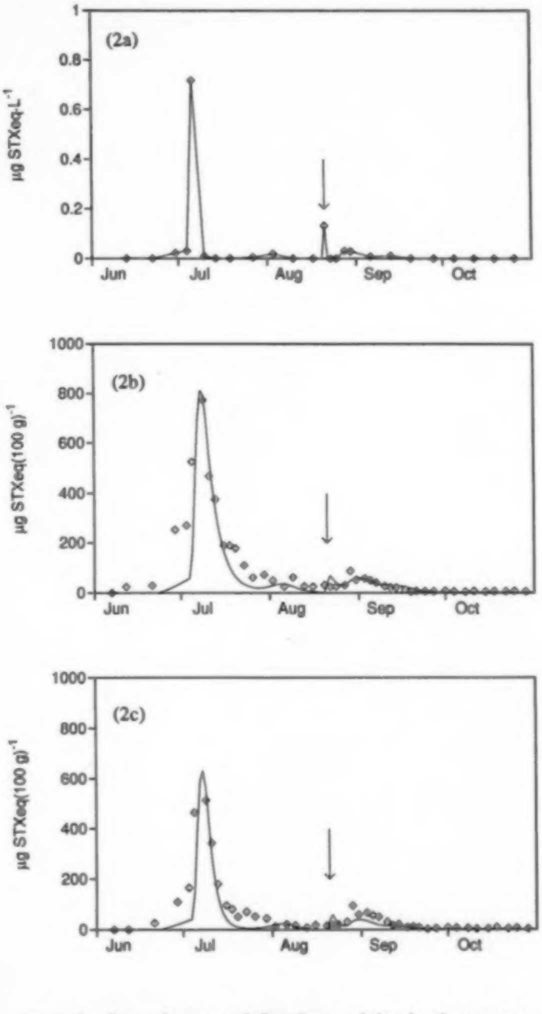
We tested this idea by carrying out a set of simulations in which the duration of the peak dominated by the measured density of 10 000 cells·L^{-1} determined on August 20 was reduced. The cell counts on August 16 and 22 were zero, and as shown in Fig. 1*a* these three points were connected by straight lines. In Fig. 2 we show the effect of assuming cell densities of zero on August 19 and 21, which is consistent with the data but corresponds to a much shorter secondary peak. It is clear that aliasing errors, namely incorrect interpolation of the algal concentrations between the dates of observation, could explain most of the discrepancy between the data and the simulation.

Both problems (1) and (2) can be resolved within the context of a one-compartment model, but problem (3) suggests the need for a two-compartment model, which could explain the retention of some toxicity for a considerable time after a bloom. Figure 3 shows the results of simulations with the two-compartment model described by [3] and [4], and although the parameter values for this model are uncertain, it clearly resolves the problem of explaining the tail of the distribution. Figures 3*c* and 3*d* show how toxins accumulate in other compartments and remain at moderately high levels long after the free or weakly bound toxin in the viscera has been eliminated.

Inverse modelling

As a test of the inference that the discrepancies between the simulation output and the mussel data might be due to errors in the values used for the toxicity in the water column, we examined the effects of inverting the model to estimate the water column toxicity from the mussel toxicity data. In general, inverting a model to obtain the forcing functions from the outputs is often difficult and sometimes impossible (Silvert 1981). Even for the system of two differential equations given in [3] and [4] it is difficult to find a robust way to estimate the ingestion term I, especially since the data give only the total toxin content $C_0 + C_1$

Fig. 2. Same simulation as in Fig. 1, except that the duration of the second peak in *Alexandrium* cell density at the point indicated by the arrows has been reduced from 6 days to 2 days, as shown in *a*. The data points are the same, but the interpolation has been changed to test the hypothesis that the high water column toxicity measured on August 20 was just a short peak possibly because of a passing *Alexandrium* patch. Simulated mussel toxicity (solid lines) is plotted against data (points) for specimens transferred from Cap Chat (*b*) and the Magdalen Islands (*c*).



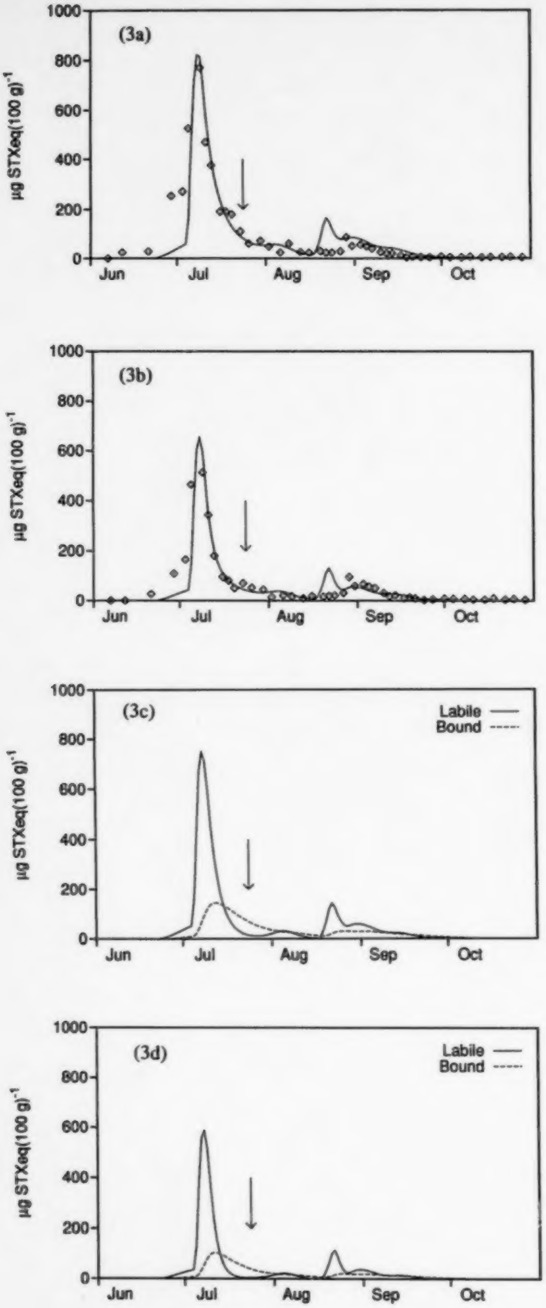


Fig. 3. Comparison of simulation results to field data on mussel toxicity for specimens transferred from Cap Chat (*a* and *c*) and the Magdalen Islands (*b* and *d*). This simulation uses a two-compartment model to resolve the discrepancy seen in the period after the initial bloom. The concentrations in the two compartments are shown in *c* and *d*, from which it is clear that the tail seen in the toxicity curves after the primary bloom in the vicinity of the arrow is due to retention in a second compartment. These simulations are driven by the same water-column toxicity data as shown in Fig. 1*a*.

and we do not have separate time series for C_0 and C_1. Fortunately, however, the simplified one-compartment model described by [5] has the much easier inverse

$$[6] \quad I = dC/dt + eC$$

by simply rearranging terms. We applied this equation to the two sets of mussel data, assuming the same filtration rate of 6.4 $L\cdot d^{-1}\cdot g^{-1}$ (wet weight) as used in the simulations, as well as the detoxification rates e of 40 and 24%$\cdot d^{-1}$ obtained by fitting the simulation model. The results (Fig. 4) show reasonable agreement with the values obtained by multiplying the cell counts by the toxicity per cell, but there are some noteworthy discrepancies.

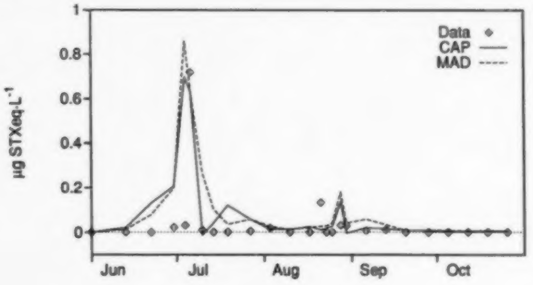
As indicated in the previous discussion, the inverse calculation shows that PSP toxins must have been present in the water column in late June, even though the measurements give only very low values. This originates from the assumption that all of the toxin in the mussels comes from the water column, hence mussels cannot become toxic without first having been exposed to toxigenic phytoplankton cells.

The inverse model shows a secondary peak in water column toxicity in late August similar to what was observed, but occurring about a week later. We have examined the possibility that this discrepancy in timing could be reduced significantly by allowing for variability in the clearance rate, but sensitivity analysis of the model indicates that both the filtration and detoxification rates would have to be reduced to unrealistically low levels to account for a delay of this magnitude (reducing these rates increases the time lag between changes in water column toxicity and the response in the mussel measurements). Seston levels are relatively low at this time, which makes any significant depression of the filtration rate unlikely.

Although there are further discrepancies during July, this is the period when it appeared that a two-compartment model would be far better, and the one-compartment model should probably not be used in the period following a bloom. Inversion of the two-compartment model is difficult and cannot be included in the present analysis.

An interesting feature of the inverse model is that at one stage during July the toxicity inferred from the CAP data becomes slightly negative, which is of course impossible. This sort of unrealistic behaviour frequently arises in inverse modelling, but the cause of the problem is easily understood. During the time interval in which this point was evaluated, the value of C fell more rapidly than could be accounted for by the detoxification rate used (in other words, the mussels detoxified faster than they would in

Fig. 4. Inverse calculation of water-column toxicity from PSP concentrations in mussels using a one-compartment model. The lines show the toxicity back-calculated from PSP toxin levels in Cap Chat (CAP) and Magdalen Islands (MAD) mussels. These are to be compared with data points representing the toxicity obtained by multiplying toxic cell concentrations by measured toxicity per cell.



seawater free of toxin), so the method computes a negative toxicity value. This is presumably due either to errors in the mussel toxicity data (which can be quite small errors) or the use of an incorrect value for *e*. Although the potential contribution of nonvisceral tissues is very small (<5% of total toxin body burden), detoxification rates may be unequal for viscera and other tissues (Bricelj et al. 1991), and thus the estimate of *e* may be slightly affected. The detoxification rate may be influenced by changes over time in the total seston load and, more specifically, by variation in the quantity and species composition of the nontoxic food source. In principle, it is also possible that detoxification rates may be sensitive to temperature changes, but the range of temperature variation (±5°C) over the bloom periods in the field were not of sufficient magnitude to explain any systematic shift in detoxification rate.

In evaluating the results of the inverse modelling, some important caveats should be noted. The values of C and $\mathrm{d}C/\mathrm{d}t$ used in [6] were obtained by linear interpolation between measured values of C, and this is not a robust procedure. In particular, on each of the dates on which C was measured the estimated value of $\mathrm{d}C/\mathrm{d}t$, and therefore of I, changes discontinuously. It would be far better to apply some sort of smoothing or averaging algorithm. The analysis shown in Fig. 4 is preliminary, and further research on how best to invert shellfish toxicity data to infer water column toxicity is currently underway.

Conclusions

It is difficult to strike an appropriate balance between simplicity and complexity in developing models. In the present case, a one-compartment model of phycotoxin kinetics provides a useful representation of toxin dynamics in mussels. Some of the data can be fit better by a simplified two-compartment model, but it is difficult to establish the validity of such a model until parameters derived from more precise data are available.

We infer that the discrepancies between the models and the data are more likely to be due to errors in the water column toxicity values used to drive the model (either because of cell counting errors, analytical error in accurate toxin determination with low toxic cell biomass, or because of aliasing problems arising from spatio-temporal patchiness and uncertainty in interpolating between discrete sampling times) than to errors in modelling the shellfish toxicity. Models of phycotoxin uptake by sedentary invertebrates can be a valuable adjunct to phytoplankton monitoring programs. Since filter-feeding species integrate the toxin concentrations to which they are exposed, by applying suitable models they can be used as time-averaging phytoplankton samplers. As shown by the discussion of inverse modelling, the development of models for back-calculating from shellfish toxicity to determine the quantity of toxins that were present in the water column can be a difficult task, but one that merits investigation.

The general theory of phycotoxin kinetics is very complex and involves many parameters describing both the physiological transfer rates of toxins within organisms and biotransformation processes. Few data are available for estimation of these parameters for field populations. Fortunately, it appears that very simple models with just one or two compartments and an aggregate representation of the many toxin components present may be sufficient for species with rapid toxin kinetics.

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Homing behavior and vertical movements of four species of Pacific salmon (*Oncorhynchus* spp.) in the central Bering Sea

Miki Ogura and Yukimasa Ishida

Abstract: Four sockeye salmon (*Oncorhynchus nerka*), two chum salmon (*O. keta*), three pink salmon (*O. gorbuscha*), and four chinook salmon (*O. tshawytscha*) with depth-sensing ultrasonic transmitters were tracked in the central Bering Sea to examine migration in the open sea. Ground speeds of maturing sockeye, chum, and pink salmon were at 0.54–0.66 m/s (0.88–1.17 fork lengths/s). Chinook salmon, probably immature fish, moved more slowly (0.34 m/s). Maturing individuals moved in particular directions and maintained their ground speeds and directions during day and night. The results also suggested that salmon had a compass orientation ability functioning without celestial information. Sockeye, chum, and pink salmon showed strong surface preferences but chinook salmon swam deeper (30–35 m) than did the other species.

Résumé : Quatre saumons rouges (*Oncorhynchus nerka*), deux saumons kétas (*O. keta*), trois saumons roses (*O. gorbuscha*) et quatre saumons quinnats (*O. tshawytscha*) ont été équipés d'émetteurs ultrasoniques avec capteurs de profondeur; on a ainsi pu les poursuivre dans le centre de la mer de Béring pour étudier leur migration en pleine mer. La vitesse sol des saumons rouges, kétas et roses en cours de maturation se situait à 0,54–0,66 m/s (0,88–1,17 longueurs à la fourche/s). Les quinnats, qui étaient probablement immatures, se déplaçaient plus lentement (0,34 m/s). Les individus en maturation se déplaçaient dans des directions particulières et maintenaient jour et nuit leur vitesse sol et leur direction. Les résultats indiquaient aussi que les saumons possèdent une capacité d'orientation qui fonctionne sans information céleste. Les saumons rouges, kétas et roses marquaient de nettes préférences pour la surface, mais les quinnats se déplaçaient à plus grande profondeur (30–35 m).

[Traduit par la Rédaction]

Pacific salmon (genus *Oncorhynchus*) are noted for their extensive ocean migrations and successful homing to their natal rivers. In the final stages of riverine migration, adult salmon use olfactory information to discriminate among the various streams (Hasler et al. 1978). The oceanic and coastal migrations are probably guided by different environmental cues, and their migratory mechanisms are less well understood.

Ocean migrations of salmonids have been studied by tagging fish for subsequent recapture and by observing seasonal abundance patterns. Tagging data showed that homing salmon may travel 40–60 km/day for several weeks in the open sea (Hartt 1966). This rate of travel is roughly equivalent to the optimal swimming speed estimated by laboratory studies (Weihs 1973; Brett and Glass 1973). However, if salmon migrate at this optimal speed during the homing migration, excellent migratory orientation must be assumed; salmon would have to move along the shortest route (great circle course) from the open sea to the respective home stream and swim continuously during day and night (Quinn and Groot 1984). If orientation is less precise, actual swimming speed would have to exceed the optimal swimming speed for the fish to achieve the observed travel rates.

However, we have no direct observations so far that salmon swim at their optimal swimming speed or not, and whether they continuously swim during day and night, and moving in a particular direction. In coastal waters, Quinn (1988) reported that the actual swimming speed of sockeye salmon (*Oncorhynchus nerka*) was about one body length/s (equivalent to the optimal swimming speed). In the open sea, Ogura and Ishida (1992) reported the horizontal movements of coho salmon (*Oncorhynchus kisutch*) in the central North Pacific but could not provide precise information on the swimming speed of the fish because of the low accuracy of the tracking vessel positioning method.

Six species of Pacific salmon are distributed in the Bering sea, though coho salmon and steelhead trout

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Table 1. Species, fork length, age of salmon tracked, and duration, direction and distance of travel of salmon tracked in the Bering Sea, 1990 and 1991.

Salmon No.	Species	Fork length (mm)	Age[a]	Duration (h:m)	Net direction[b]	Net distance (km)[c]	Gross distance (km)[d]
9006	Sockeye	582	2.3	91:00	97	140.6	203.0
9102	Sockeye	631	2.3	55:00	64	97.8	118.5
9103	Sockeye	640	1.3	15:40	97	7.2	33.1
9106	Sockeye	648	1.3	54:30	313	44.4	63.4
9003	Chum	638	0.4	31:30	114	21.9	52.5
9007	Chum	688	0.4	42:30	264	123.1	131.7
9101	Pink	497	0.1	54:15	259	69.9	82.8
9108	Pink	476	0.1	15:15	276	21.0	30.0
9109	Pink	520	0.1	54:30	330	31.5	150.8
9005	Chinook	638	1.3	80:00	189	102.4	120.2
9104	Chinook	775	1.3	55:40	144	18.5	61.1
9105	Chinook	800	1.4	54:20	59	23.7	52.8
9107	Chinook	650	1.3	50:10	224	43.7	67.8

[a] The number preceding the point is the number of winter annuli spent in fresh water and the number following the point is the number of winter annuli spent in the ocean (Koo 1962).

[b] The direction from the point of release to the point where the fish was lost, in degrees relative to geographic north.

[c] The shortest distance from the point of release to the point where the fish was lost.

[d] The total distance tracked.

(*Oncorhynchus mykiss*) are scarce. The salmon originate from several coastal regions, and local stocks are well mixed in the Bering Sea (Royce et al. 1968). The present study describes the horizontal movements of four species of Pacific salmon (sockeye, chum, *Oncorhynchus keta*; pink, *Oncorhynchus gorbuscha*; and chinook, *Oncorhynchus tshawytscha*) in the Bering Sea by ultrasonic telemetry using a more stable positioning method, Loran (Long Range Navigation) C rather than the NNSS (Navy Navigation Satellite System) used by Ogura and Ishida (1992). We estimate the travelling speeds and examined the migratory orientation of Pacific salmon. To better understand the vertical movements of salmon, we used depth sensitive transmitters to provide direct information about the vertical distribution of Pacific salmon in the open sea.

Methods

The tracking was conducted by the Japanese salmon research vessel *Shin-riasu maru* (471 gross tonnes, 48 m length) between 23 June and 10 July 1990 and 20 June and 10 July 1991 in the international waters of the central Bering Sea (Fig. 1). All salmon used were caught by surface longline and were designated by four digit numbers; the two first digits indicated the year of the tracking and two latter the serial number of tracking in the year (Table 1).

The depth sensing (pressure-sensitive) transmitter (65 mm long × 15 mm diameter, weighing 11 g in water, Vemco Ltd., Dartmouth, Nova Scotia) calibrated at the research point was attached externally. Four rubber bands (two in front and two in the back) were fastened around the transmitter, and two nylon cable ties (Dennison Cable Tie Products, Dennison Transoceanic Corp., Tokyo) were threaded through the rubber bands at each end of the transmitter. The transmitter was mounted to the fish by running the cable ties through the musculatum immediately anterior to the fish's dorsal fin. At the time of transmitter attachment, fork length of each fish was measured and a few scales were taken for age determination. The fish were out of the water for approximately 1 min during this procedure and we did not use anaesthesia.

The fish were allowed 10–15 min to calm down before release. The telemetered fish was followed using a directional hydrophone mounted on the tracking vessel. The output of a digital signal on time and swimming depth from the receiver (Vemco Ltd.) was recorded on a personal computer diskette at 5-s intervals. The vessel's position was determined with Loran C, recorded at intervals of 15 min, and used to approximate the fish's position for calculation of distance travelled. Vertical profiles of temperature to 250 m were measured immediately before and after the tracking period of each fish. Expendable bathythermographs deployed approximately every 12 h provided additional ocean temperature profiles.

Ground speeds were calculated by distances moved in each 15-min intervals. The directional speed was defined by total net distance (shortest distance from point to point) per total duration. Daytime and nighttime were divided at the sunset and the sunrise times, respectively. Average swimming depths during every minute were calculated for analysis of depth frequency and average depth. The rate of vertical movement (vertical distance per second) was calculated for dives or ascents of more than 10 m using 5-s interval depth data.

Fig. 1. The area in the Bering Sea where Pacific salmon tracking took place and horizontal movements of 13 Pacific salmon tracked. Solid and dotted lines in horizontal movement indicate tracks in daytime and in nighttime, respectively. In the Bering Sea at summer, nighttime is only about 7 h/day.

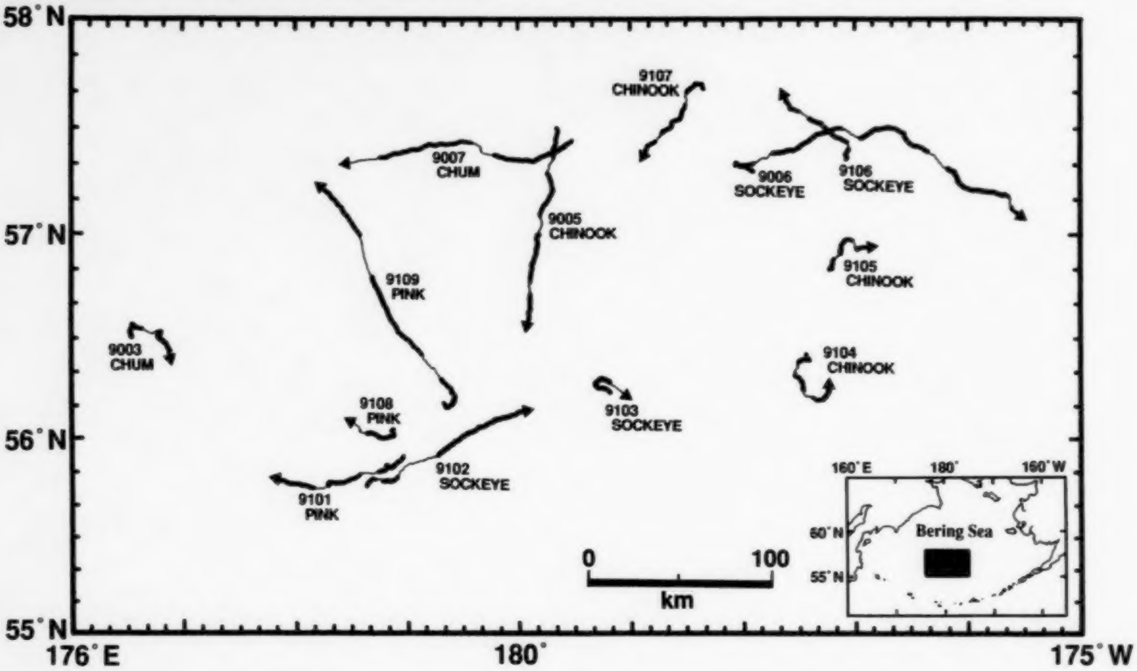


Table 2. Horizontal movements of four Pacific salmon species tracked using ultrasonic transmitters.

Salmon species	Directional speed (km/day)[a]	Average ground speed (m/s) (fork length/s)[b] Overall	Day	Night
Sockeye	32.2	0.54 (0.88)	0.54	0.52
Chum	47.0	0.66 (0.98)	0.69*	0.59*
Pink	43.1	0.59 (1.17)	0.59	0.60
Chinook	18.8	0.34 (0.49)	0.34	0.35

Note: *, significant difference between daytime and nightime average ($P < 0.05$).

[a] Calculated by dividing net distance by duration.

[b] Calculated by dividing gross distance by duration.

Results

Tracks of four sockeye, two chum, three pink, and four chinook salmon are reported. The duration of tracks from release to termination ranged from 15 to 91 h (Table 1). Total tracking times for each species were 216 h for sockeye, 74 h for chum, 124 h for pink, and 240 h for chinook salmon. Therefore numbers of available data of the ground speed and the average swimming depth were 295 and 4209 for the shortest tracked species, chum. Tracking times were limited mainly by the restriction of research schedules or the change of the weather condition. Tracks of one sockeye salmon 9006 and two chinook salmon, 9005 and 9107, were terminated because the depth indications exceeded the tolerance of the transmitter (350 m). We assume that the transmitters became separated from the fish or that the fish died. Data during these final dives were eliminated from analysis.

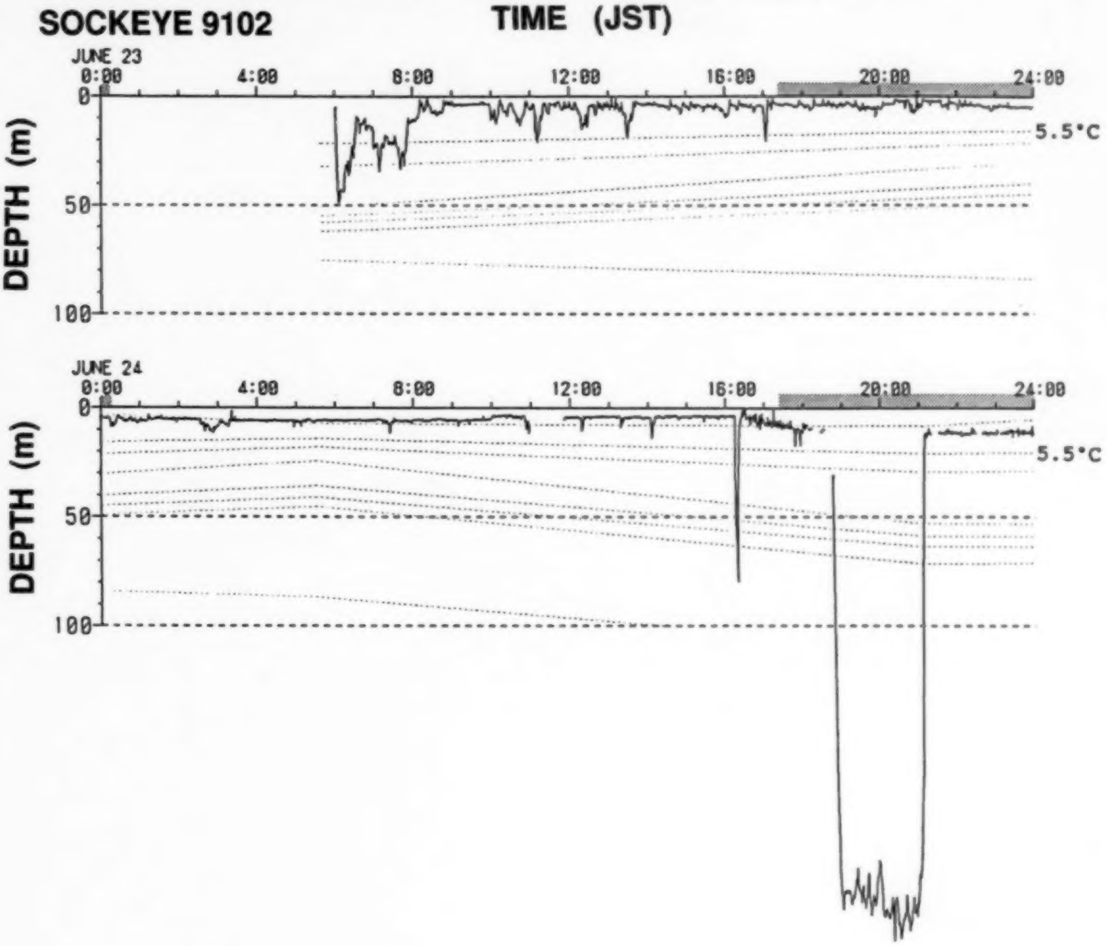
Judging from their ages and fork lengths (Table 1), the pink salmon must have been maturing fish (Takagi et al. 1981) and the sockeye salmon and chum salmon were also presumed to be maturing (French et al. 1976; Neave et al. 1976). On the other hand, the chinook salmon used may have been immature, because most chinook salmon distributed in the Bering Sea in late June and July were reported to be immature (Major et al. 1978). In addition, all 34 chinook salmon greater than 600 mm fork length (638–796 mm, average = 698 mm) caught during the same tracking research cruises in the 2 years were immature (unpublished data).

Horizontal movements

Pink salmon moved in some direction, while other species showed much individual variation (Table 1, Fig. 1). These salmon, especially the sockeye, chum, and pink, maintained relatively fixed directions while tracked (Fig. 1). However during the initial hours of their tracks some fish headed in different directions from the final directions. For example, sockeye salmon 9006 swam on a westerly course for first 4 h then turned east. Pink salmon 9109 moved southeastward during the first 3 h after release and began to change its direction counterclockwise to northwestward.

Average ground speeds of sockeye, chum, and pink were more than 0.5 m/s and equivalent to about 1 fork

Fig. 2. The vertical movement of tracked sockeye salmon 9102 during the 1st and 2nd day of tracking. Shaded time zones indicate nighttime. Dashed horizontal lines represent 0.5°C isotherms. JST, Japan standard time.

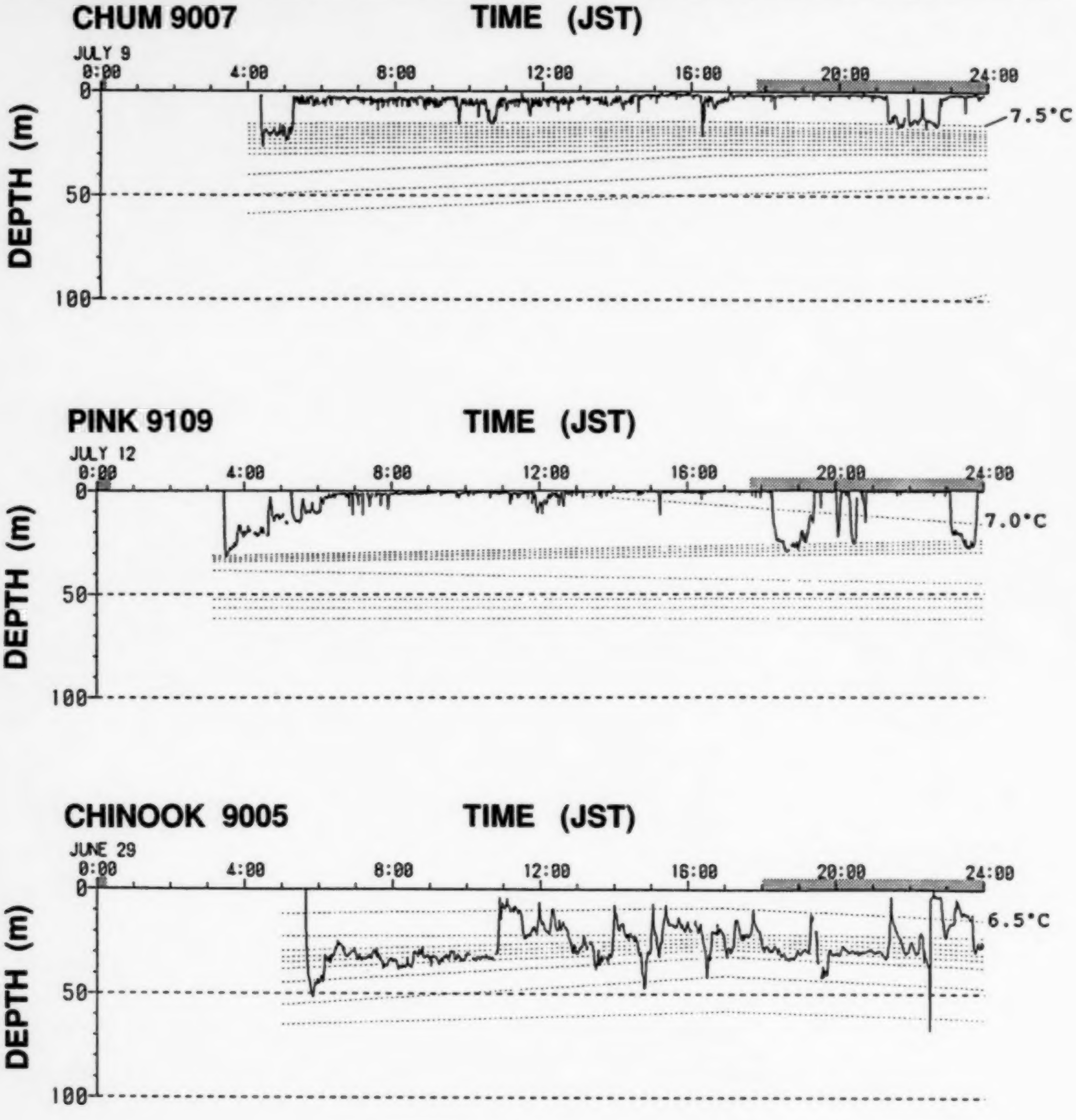


length/s (Table 2). On the other hand, the average ground speed of chinook salmon was slower than the other three species and the directional speeds was also considerably less than those species (Table 2). Average ground speeds in daytime were faster than in nighttime for chum salmon (*t*-test, $P < 0.05$; Table 2), but there was no significant difference for the other three species. The difference in daytime and nighttime ground speeds was not consistent among individual fish within species. Moreover, the ground speeds of individual fish varied but no regular daily patterns of change in ground speeds and distance traveled were observed (Fig. 1). The variation in ground speed during the track of each fish was not so large, but sockeye 9102 showed a obvious acceleration of the ground speed at dusk of the second day of tracking from average 0.25 m/s to average 1.17 m/s (1.9 fork length/s). The fish continued to swim at this speed during the following 20 h.

Vertical distribution

All salmon tracked showed an initial dive immediately after release (Figs. 2, 3). Most initial dives were to 25–52 m in depth and seemed to be restricted to the water above or in the thermocline, except for the deepest initial dive of chinook salmon 9107 (124 m in depth). Following the initial dive, fish ascended slowly or made brief dives and ascents in the thermocline (Figs. 2, 3). The rate of vertical movement during initial dives was not so fast and the maximum depth was usually recorded at another time in the track. Therefore we did not eliminate the data from these initial dives. After initial dives, most vertical excursions of sockeye, chum, and pink salmon were above or down into the thermocline which was at about 20–40 m in most areas. In most vertical excursions by sockeye, chum, and pink salmon, the fish started to ascend immediately following the dive, resulting in a V-shaped time-depth profile (Figs. 2, 3).

Fig. 3. Vertical movements of tracked chum salmon 9007, pink salmon 9109, and chinook salmon 9005 of the 1st day of tracking. Shaded time zones indicate nighttime. Dashed horizontal lines represent 0.5°C isotherms. JST, Japan standard time.

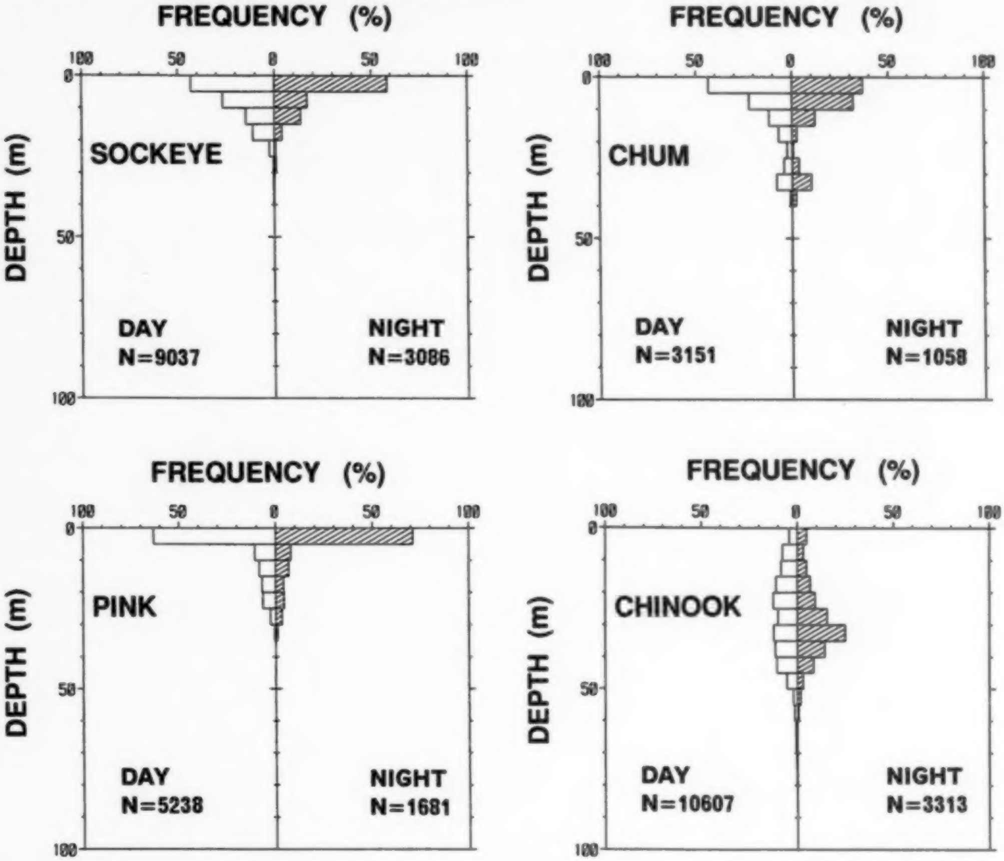


On the other hand, chinook salmon swam in the thermocline with occasional vertical excursions toward the surface and the deeper layer. These vertical excursions of chinook salmon were also V shaped or reverse V shaped (Fig. 3).

Although the average depths of sockeye, chum, and pink salmon were less than about 20 m, swimming depths of chinook salmon averaged 29.1 m (Table 3). The vertical distributions of the four salmon species were generally restricted to the upper 50 m of the water column (Fig 4). Sockeye (70% of the time during day, 76% at night) and pink salmon (75% day, 79% night) spent most of time in the upper 10 m of the water column. Chum salmon also swam near the surface (66% day and 68% night), in the upper 10 m, but spent 9% of the day and 12% at night in the 30–40 m depth layer. On the other hand, chinook salmon spent only 12% of the day and 8% at night in the upper 10 m, and 40% of the day and 51% at night at 30–50 m.

For sockeye and chinook salmon, average swimming depths in daytime tended to be shallower than in nighttime, that of pink salmon was deeper in daytime than at night, and

Fig. 4. Depth distributions expressed as a percentage of records averaged during 1 min of four Pacific salmon species. *N*, number of average swimming depth data used.



there was no significant difference for chum salmon (t-test, $P < 0.05$; using the logarithmically transformed data for sockeye, chum, and pink salmon $X' = \log(X+1)$; Table 3). However, there were no regular differences within individuals for average depth and depth frequency at day and night of each day of the tracking. Also, no consistent daily pattern of vertical movements was observed in individual fish.

Average rates of vertical movement were similar for all species (Table 3), and ranges of average rate of vertical movement of individual fish were 0.08–0.22 m/s and the ascending speeds were 0.08–0.33 m/s, including four unusually rapid dives and ascents by sockeye salmon. The rates of vertical movement during initial dives were from 0.08 to 0.43 m/s and only two fish recorded their fastest rate during initial dives.

Discussion

Ground speeds of maturing sockeye, chum, and pink salmon we tracked were consistent with the rates of conventionally tagged salmon (Hartt 1966) and were close to the optimal swimming speeds (Weihs 1973). Our tracking data showed clearly that maturing salmon moved in fixed directions and did not significantly change their ground speeds and directions during day and night. Therefore, the net direction of fish tracked was supposed to be the direction of the natal stream or the direction of the optimal travel course determined by a bicoordinate map sense of fish (Quinn and Groot 1984); however, we could not confirm this yet. Further studies should focus on the migratory course during longer periods and the degree of bicoordinate navigation.

Quinn and Groot (1984) proposed a magnetic or celestial compass for maintaining the appropriate compass direction for homing. Experimental studies in fresh water documented solar and magnetic compass orientation of salmon (Quinn 1980; Quinn et al. 1981; Quinn and Brannon 1982; Quinn and Groot 1983). Biogenic magnetic materials, which may be used as a navigation aid, were found in sockeye (Walker et al. 1988; Mann et al 1988; Sakaki et al. 1990), chum (Ogura et al. 1992), chinook salmon (Kirschvink et al. 1985), and Atlantic salmon, *Salmo salar* (Moore et al. 1990). Salmon we tracked maintained their ground speeds and directions during the night and therefore did not reduce the degree of orientation during periods when they could not use a solar compass. Furthermore, sockeye salmon 9102

Table 3. Average swimming depth and rate of vertical movement of each salmon species.

	Average swimming depth (m)			Average rate of vertical movement (m/s)	
Species	Overall	Day	Night	Dive	Ascent
Sockeye	9.9	8.1	15.1	0.19 (0.06–2.19)	0.16 (0.00–0.83)
Chum	10.2	10.1	10.4	0.16 (0.06–0.39)	0.14 (0.05–0.44)
Pink	5.9	6.2	5.1	0.16 (0.03–0.46)	0.16 (0.03–0.44)
Chinook	29.1	28.8	30.0	0.12 (0.02–0.63)	0.14 (0.03–0.75)

Note: Values in parentheses indicate the range of rate of vertical movement.

maintained its ground speed and swimming direction while swimming at a depth of 240 m (Fig. 2). Those findings suggest that the salmon were using a directional cue that functions without light stimulus in the open sea.

Net directions of sockeye, chum, and pink salmon tracked were categorized roughly westward and eastward, namely Asian and North American continents, and showed the mixed distribution of local stocks in the central Bering Sea in summer. An interesting finding was that some fish varied their travel direction during the first several hours of tracking and then seemed to fix the direction. After attachment of an external transmitter it took 24 h for saithe (*Gadus pollachius* Virens) to resume a normal heart beat rate (Mohus and Holand 1983); this period was reduced if the fish was kept submerged during the transmitter attachment. Our salmon may have been disturbed by handling for several hours after release. However, we could not estimate the precise duration of disturbance and did not eliminate data immediately after release.

Our ground speed data did not take currents into consideration. Although we were unable to obtain information on current in the research area, we concluded that there is no large effect on the swimming behavior by the current because the currents speed in the Bering Sea Basin is in general a few centimetres per second (Ohtani 1973). We must also consider whether the externally attached transmitter reduces swimming performance. Mellas and Haynes (1985) reported that increased drag from external transmitters may cause fish to reduce or discontinue swimming. However, we assumed that the effects of transmitters were minimal because one sockeye moved continuously at 1.2 m/s (= 4.2 km/h) during 20 h, which was close to the fatigue swimming speed (Brett 1983).

Compared with the other salmon species, chinook salmon showed the slowest ground speed. The reason for this slow speed may be that they were immature and were not migrating home. Some chinook varied their direction throughout the track or travelled southward where no natal streams were expected. However, the slow ground speed did not necessarily mean that chinook swam slowly or were inactive. The fish were followed at a distance of up to 200 m and the tracking technique used in this study was not capable of detecting small-scale changes in the horizontal movements by the fish.

Like coho salmon (Ogura and Ishida 1992), the three maturing salmon species (sockeye, chum, and pink) inhabited near-surface waters, mostly shallower than 20 m, and their vertical movements seemed to be restricted by the thermocline. These results generally agree with results of previous studies using specialized fishing gears (Manzer 1964; Manzer et al. 1965; Machidori 1966; French et al. 1976). In contrast, chinook salmon had an obviously deeper swimming depth and upward vertical excursions were only occasionally observed. This may explain the frequent bycatch of chinook salmon in trawl fisheries (Major et al. 1978; Allen and Smith 1988). Deeper swimming depth of chinook probably was not related to feeding habits because coho salmon diets are similar to those of chinook (Brodure 1990). Moreover maturity stage also probably was not a determining factor affecting the vertical distribution of chinook, because immature steelhead trout show the strongest surface preference among the Pacific salmon (Ogura and Arai 1992). This clear difference in the vertical distribution of chinook salmon from other salmon species should be taken into account when comparing abundance data obtained with surface gears.

Although summarized data of vertical movements of fish tracked in this study showed some differences in average swimming depth between daytime and nighttime, individual salmon did not show regular daily vertical movements, as did coho salmon (Ogura and Ishida 1992). While catch by specialized fishing gears showed daily patterns in swimming depth of migrating salmonids (Manzer 1964; Machidori 1966; Pearcy et al. 1984), these patterns may occur because of avoidance of gear and poor operation of gears at the deeper depth.

Rates of vertical movement of salmonids in the coastal waters and the open sea were reported to be 0.05–0.33 m/s (Ichihara et al. 1975; Ichihara and Nakamura 1980; Westerberg 1982; Soeda et al. 1985, 1987; Shimamura 1987; Quinn et al. 1989; Ogura and Ishida 1992; Yano and Nakamura 1992). Although rates of initial dives of coho and chum were more rapid than those of usual dives (Ogura and Ishida 1992; Soeda et al. 1987), rates of initial dives in this study were less than 0.44 m/s and were within the range of the rate of usual vertical excursions. We conclude that Pacific salmon generally move vertically at a slow rate and that there was no substantial difference on the rate of vertical movement among the species.

In conclusion, in the open sea three species of maturing Pacific salmon swam at an average of 0.54–0.66 m/s (0.88–1.17 fork lengths/s). Maturing individuals moved in particular directions and maintained their ground speeds and directions during day and night. Therefore our tracking data partly confirmed an hypothesis on salmon homing ability proposed by Quinn and Groot (1984) based on tagging data.

Acknowledgments

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Spawning energetics of Arctic cod (*Boreogadus saida*) in relation to seasonal development of the ovary and plasma sex steroid levels

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Abstract: Gonadal development in Arctic cod starts in August in high Arctic Canada. Male gonads develop at a faster rate than female gonads initially, and reach maximum size about two months prior to females. Under laboratory conditions (at 1.0°C), female Arctic cod became ripe and started to spawn in mid-February, which concurred with field observations. Ripe gonad weight increased with body size; mean gonadosomatic index (GSI) of ripe females was 47.7 %, and potential fecundity averaged 26 500 eggs. At the time when ripe females started to spawn, some females were still unripe (GSI = 20.2 %). Their eggs were not hydrated, and these fish had significantly higher levels of sex steroids (estradiol, 11-ketotestosterone, and testosterone) in their blood than ripe fish. Arctic cod are repeat spawners, at least under laboratory conditions, in spite of the large amount of energy allocated to reproduction (306 kJ lost from liver and muscle during gonadal development and 125 kJ as eggs during spawning). A high energy investment into reproduction, for a fish that matures at an early age, may be balanced against high predation rates on this species during the open-water season in the Canadian high Arctic.

Résumé : Le développement gonadique commence en août chez le saïda franc de l'Extrême-Arctique canadien. Les gonades mâles se développent au départ plus vite que les gonades femelles, et atteignent leur taille maximum environ deux mois avant ces dernières. Dans des conditions de laboratoire (à 1,0°C), le saïda franc a atteint la maturité et a commencé à frayer à la mi-février, ce qui correspondait aux observations de terrain. Le poids des gonades à maturité augmentait en fonction de la taille du corps; l'indice gonadosomatique moyen (IGS) des femelles à maturité était de 47,7%, et la fécondité potentielle était en moyenne de 26 500 oeufs. Au moment où les femelles matures ont commencé à pondre, certaines femelles n'étaient pas encore matures (IGS = 20,2%). Leurs oeufs n'étaient pas hydratés, et ces poissons présentaient des concentrations sanguines de stéroïdes sexuels (estradiol, 11-cétotestostérone, et testostérone) nettement plus élevées que les poissons matures. Le saïda franc est un géniteur multifraye, au moins dans des conditions de laboratoire, malgré la grande quantité d'énergie qui va à la reproduction (306 kJ tirés du foie et des muscles pendant le développement gonadique et 125 kJ sous forme d'oeufs pendant la ponte). Le grand investissement énergétique dans la reproduction, chez un poisson dont la maturation est précoce, peut contrebalancer les taux élevés de prédation que subit cette espèce pendant la saison d'eaux libres dans l'Extrême-Arctique canadien.
[Traduit par la Rédaction]

Introduction

Spawning of Arctic cod (*Boreogadus saida* Lepechin, 1774) has never been directly observed in the Canadian Arctic, where this species is a key element in the marine food web (Hobson and Welch 1992; Welch et al. 1992). Little is known about the reproductive ecology and physiology of this species, possibly because Arctic cod typically spawn under the ice in the darkness of the Arctic winter (Rass 1968). Most information on spawning of Arctic cod is from Russian waters (e.g., Nikol'skii 1954; Baranenkova et al. 1966; Rass 1968), and to a lesser extent from the Alaskan Arctic (Craig et al. 1982) and northwest Atlantic (Lear 1979, 1980). Gonadal maturation starts in August

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(Bain and Sekerak 1978; Hop et al. 1995), with males developing gonads more rapidly than females (Nikol'skii 1954; Hop et al. 1995). At the time of early gonadal development, Arctic cod have been observed in large aggregations or schools in nearshore, shallow waters (Andriyashev 1954; Ponomarenko 1968; Bain and Sekerak 1978; Craig et al. 1982; Welch et al. 1993; Hop et al. 1995). Spawning occurs from December to the end of March in the Barents Sea (Rass 1968), with peak spawning in January and February (Andriyashev 1954); it has been inferred that spawning occurs at a similar time in Alaskan waters (Craig et al. 1982). Maturation of females generally occurs at 3 years of age, whereas males frequently reach maturity at 2 years (Lear 1979; Craig et al. 1982); in Russian waters maturity may be delayed until 4 years of age (Nikol'skii 1954). Female spawning output has been reported to be between 9000 and 21 000 eggs. Arctic cod eggs are the largest among gadid fishes (Andriyashev 1954; Matarese et al. 1989).

Laboratory studies of reproduction in Arctic cod have focused on artificial fertilization and embryonic and larval development of Arctic cod from Russian waters (Doroshev and Aronovich 1974; Aronovich et al. 1975; Altukhov 1981), especially the effects of temperature, salinity, and food consumption. Recently, we observed spawning of captive Arctic cod obtained from the Canadian high Arctic, and investigated incubation time, hatching, and growth and development of larvae (Graham and Hop 1995). The present study complements the latter study by investigating aspects of seasonal gonadal development and function, energy allocation, and output. We hypothesize that Arctic cod invest higher amounts of energy into reproduction than other gadids, and may thus compromise post-spawning survival with reproductive output.

Materials and methods

Fish samples

Arctic cod were caught in trap nets in Resolute and Allen bays, Cornwallis Island, N.W.T., during late-July–September, 1988–1991. Fish ($n = 52$, size range 154–276 mm) were packed and transported (Graham and Hop 1995) from the Arctic to two laboratory facilities: Oceans Sciences Centre (OSC), Memorial University of Newfoundland, St. John's (in 1988); and Vancouver Aquarium (VAQ) (in 1990, 1991). In the laboratories, fish were kept in two, 300-L tanks at OSC and one, 800-L tank at VAQ. Water temperatures were 0.2 ± 0.6°C (OSC) and 1.0 ± 1.0°C (VAQ), with respective salinities of 30.5 ± 1.5‰ and 29.0 ± 1.0‰. Fish were fed frozen capelin (*Mallotus villosus* Müller, 1777) at OSC and frozen krill (*Euphausia pacifica* and *E. superba*) at VAQ, three to five times per week. Krill is a relatively soft-shelled prey with similar energy content (±SE) as capelin (krill = 4.898 ± 0.004 kJ/g wet weight; capelin = 5.039 ± 0.057 kJ/g wet weight).

Tissue samples

Fish were killed at different stages of gonadal development (July–February). Early samples (late-July–September) were fish caught directly from Resolute and Allen bays, whereas later sources were fish that had been kept at OSC (mid-December) and VAQ (December–February). Fish were measured, weighed, and sexed before livers and gonads were dissected out and weighed wet. Carcass weight was determined after removal of internal organs. For samples in December–February, carcass weight was not determined, although we estimated it by subtracting the weight of other organs (gonad, liver, intestines) from total body weight. The unknown weight of stomach content (krill) was included in carcass weight.

Tissue indices

From these measurements we calculated a gonad weight/body weight index (GSI), the gonadal development index that is most frequently reported in the literature (e.g., Crim and Glebe 1990; Smith et al. 1990; Kjesbu et al. 1991):

Gonadosomatic index (GSI) = (gonad weight/whole body weight) × 100.

Also calculated were weight/fork length indices for liver and carcass: hepatosomatic index (HSI) = (liver weight/fork length) × 100; somatosomatic index (SSI) = (carcass weight/fork length) × 100.

We needed to know the changes in these organs over time to calculate energy loss during gonadal development, and we related these indices to fork length (FL, mm) instead of whole body weight to avoid auto-correlation between tissue weights and total body weight. These indices were standardized to the same fork length (245 mm, the mean length of ripe females; Table 1) based on samples collected in July/August. The following linear regressions (with $p < 0.0003$ for all slopes) were applied in length corrections for females (F) and males (M):

$$\text{F-HSI}(\%) = 0.029 \cdot \text{FL} - 2.623, \; r^2 = 0.550$$
$$\text{M-HSI}(\%) = 0.026 \cdot \text{FL} - 2.417, \; r^2 = 0.526$$
$$\text{F-SSI}(\%) = 0.183 \cdot \text{FL} - 13.883, \; r^2 = 0.902$$
$$\text{M-SSI}(\%) = 0.158 \cdot \text{FL} - 10.845, \; r^2 = 0.813$$

Tissue analyses

Gonad, liver and carcass (without otolith) were dried at 60–70°C to constant weight (3–5 days). Homogenized dried samples (1.0 g) were taken for ashing (595°C) and caloric determinations (Adiabatic oxygen bomb calorimeter, model 1241) with results expressed in Joules (1 J = 0.2389 cal). In addition, total protein was determined by the Kjeldahl nitrogen procedure (Bradstreet 1965), and total lipids were determined by chloroform/methanol extraction (Folch et al. 1957).

Relative composition of lipid classes was determined on an Iatroscan TH-10 TLC/FID analyzer using S-III Chromarods. This method involves separation of lipids on silica-coated rods and their subsequent detection by a flame ionization detector (Ackman 1981). Fresh gonadal and liver tissues were stored refrigerated in chloroform/methanol (2:1 by volume), and lipids were extracted by the Folch et al. (1957) method prior to spotting onto the chromarods. The rods were developed for 60 min in a solvent system of benzene – chloroform – acetic acid – methanol (49:8:0.3:0.2 by volume). Known standards of monoglycerides, diglycerides, triglycerides (glycerides = acylglycerols), phospholipids, cholesterol, cholesterol esters, free fatty acids, and wax esters were run under identical conditions as the samples for peak identification. All standards were laboratory

Table 1. Gonad composition (mean ± SE) of ripe and unripe Arctic cod, held for 5 months at the Vancouver Aquarium prior to spawning (14 February 1992).

Maturity/sex	*n*	Fork length (mm)	Fish weight (g)	Gonad weight (g)	GSI (%)	Water (%)	Protein (%)	Lipids (%)	Ash (%)	Other (%)	Energy ($J \cdot g^{-1}$)
Ripe females	17	245±2	141.2±5.8	67.5±3.2	47.7±0.9	92.0±0.2	4.1±0.2	1.2±0.1	0.3±0.0	2.4±0.0	1836.2±58.1
Unripe females	5	249±5	91.0±4.9	20.2±0.8	22.3±0.9	76.5±0.9	15.7±0.6	4.5±0.2	0.9±0.1	2.3±0.1	5781.8±235.0
Ripe male	1	233	77.4	23.0	29.7	86.3	9.8	1.6	1.7	0.6	2903.3

Note: Gonadosomatic index (GSI), levels (%), and energy relate to wet weight. "Other" material was determined by subtraction and thus includes sample loss in analyses.

made except for the standard for wax esters which was a lipid extract of *Calanus hyperboreus*. This Arctic copepod species contains >80% of this lipid class during July–November (Lee 1974), although other non-polar lipids could also be present. Data were analyzed with a Shimadzu EZ Chrom Data System.

Fecundity and egg size

Eggs were stripped when fish first started to spawn in aquaria (mid-February). Ripe fish could be easily stripped by applying pressure to the abdomen. Eggs remaining after stripping of ripe fish and gonads of unripe fish were removed by dissection. Potential fecundity (mean number of eggs produced by ripe females) was determined from 1.0-g subsamples of gonads, with unstripped eggs included in gonad weight. Diameters of 22–25 fresh eggs were measured to the nearest 0.01 mm with calipers. Eggs measured were not water hardened as they had been dry stripped from gonads.

Energy costs

Spawning energy loss (TEG) was determined for females (F) and males (M) from the weight of ripe gonads and their weight-specific energy content:

$$\mathrm{TEG\ (kJ)} = \mathrm{GW} \cdot \mathrm{EG} \cdot 10^{-3}$$

where GW is gonad weight (g) and EG is the energy content of gonads ($J \cdot g^{-1}$) (Table 1).

Energy losses from liver (TEL) and carcass muscle (TEC) during gonadal development were estimated for females and males from indices (HSI, SSI) in July/August (time_1) and February (time_2) and the energy content of these tissues (EL, EC). Weight-specific energy content (mean ± SE) of liver and carcass in July/August were

$$\mathrm{EL}_1\ (\mathrm{F;\ M}) = (21\,956.8 \pm 343.6\ ;\ 24\,066.5 \pm 229.2)\ J \cdot g^{-1}$$

$$\mathrm{EC}_1\ (\mathrm{F;\ M}) = (4419.0 \pm 55.2\ ;\ 4230.9 \pm 65.1)\ J \cdot g^{-1}$$

Energy losses within liver and muscle tissues were not determined directly, although we estimated energy lost from livers based on their lipid and protein contents (Table 2) and energy values of lipids (36.2 $kJ \cdot g^{-1}$) and protein (23.6 $kJ \cdot g^{-1}$) (Brett and Groves 1979; Brafield and Llewellyn 1982). Estimated mean energy content of livers in February were

$$\mathrm{EL}_2\ (\mathrm{F;\ M}) = (11\,934.6\ ;\ 21\,412.8)\ J \cdot g^{-1}$$

assuming that no change occurred in their energy content within the last month prior to spawning. The energy content of muscle was assumed to be constant for the whole development period (i.e., $\mathrm{EC}_2 = \mathrm{EC}_1$). This may have lead to underestimates of the total energy loss, but potential changes were unfortunately not accounted for.

Total energy losses from liver and carcass were determined for each fish:

$$\mathrm{TEL\ (kJ)} = [(\mathrm{HSI}_1 \cdot \mathrm{EL}_1) - (\mathrm{HSI}_2 \cdot \mathrm{EL}_2)] \cdot \mathrm{FL} \cdot 10^{-5}$$

$$\mathrm{TEC\ (kJ)} = (\mathrm{SSI}_1 - \mathrm{SSI}_2) \cdot \mathrm{EC}_1 \cdot \mathrm{FL} \cdot 10^{-5}$$

where FL is fork length (mm) (Table 1); calculations were carried out separately for females and males. Total energy loss during gonadal development was TED = TEL + TEC.

Sex steroids

We measured the plasma levels of sex steroids in Arctic cod as an endocrine index of gonadal function. Levels were determined in captive ripe and unripe fish at the time of first spawning (mid-February). Blood samples were taken from the caudal blood vessel, centrifuged for 20 min before the plasma layer was removed, conserved with methiolate, and then immediately frozen. Concentrations of the sex steroid hormones estradiol [E_2], 11-ketotestosterone [11-KT], and testosterone [T] were determined by radioimmunoassay after ether extraction (Van Der Kraak et al. 1984; Van Der Kraak et al. 1990; Wade and Van Der Kraak 1991). As T, by two different biosynthetic pathways, is a precursor for both E_2 and 11-KT (Fostier et al. 1983), we determined the ratios of [E_2] and [11-KT] to [T] to assess possible differences between ripe and unripe females.

Results

Tissue changes during gonadal development

Gonadal development of Arctic cod began in August, with male gonads initially developing at a faster rate than female gonads (Fig. 1*a*). Male gonads reached full size by early December, about 2 months prior to females. Gonads of ripening females developed exponentially (regression $p = 0.0001$; $r^2 = 0.981$). Arctic cod, held at VAQ, became ripe and started to spawn by mid-February. The GSI of ripe female fish was high (mean = 47.7 %, range 38.5–52.5%; Table 1). However, at the time of spawning, 23% of the females were still unripe and had significantly lower GSI (22.3%; *t*-test, $p = 0.0001$) than ripe females. Body weight with gonads removed, or length of unripe fish was not significantly different from those of ripe fish (*t*-tests, $p >$

Fig. 1. Gonadosomatic, GSI (*a*), hepatosomatic, HSI (*b*), and somatosomatic, SSI (*c*), indices for male and female Arctic cod during the season of gonadal development. Numbers next to symbols are number of fish and vertical bars indicate SE (with only one of the bars shown on symbols that are close together, and no bars shown if they are smaller than the symbol size). Data from July and August are from the Resolute Bay area (Hop et al. 1995), data from mid-September and November are from the Beaufort Sea, Alaska (Craig et al. 1982), and data from December to February are from Resolute Bay fish held at the Vancouver Aquarium and the Ocean Sciences Centre.

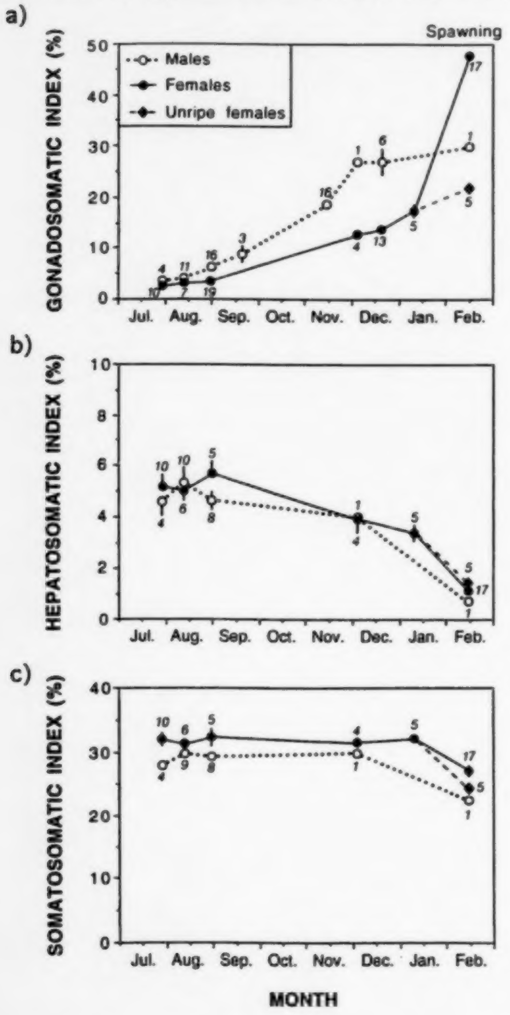


Table 2. Total protein and lipids, and relative composition of lipid classes, of gonads and livers of Arctic cod early (August) and late (December/January) in the gonadal development period.

	August		December/ January	
Organ/ lipid class	Females (%)	Males (%)	Females (%)	Males (%)
Gonad	*n* = 6	*n* = 8	*n* = 3	*n* = 1
Total protein	11.9±0.4	12.4±0.1	20.4±1.1	13.5
Total lipids	4.5±0.2	3.6±0.1	5.5±0.4	2.7
TG	2.9±0.3	6.5±2.8	4.4±0.9	5.1
WE	37.5±1.4	30.6±4.6	—	—
CH	2.7±0.4	5.8±0.7	1.6±0.4	3.7
PL	51.7±1.3	48.8±3.0	94.0±1.1	91.2
FFA	5.4±0.4	8.2±1.6	—	—
Liver	*n* = 9	*n* = 7	*n* = 3	*n* = 1
Total protein	5.6±0.5	4.6±0.3	11.6±1.3	5.2
Total lipids	59.2±3.3	65.1±2.5	26.6±4.4	57.2
TG	96.6±1.3	96.0±0.6	89.3±2.4	98.8
WE	1.5±0.6	2.2±0.6	—	—
CH	—	—	0.9±1.1	0.1
PL	1.3±0.3	1.2±0.2	9.7±3.2	1.2
FFA	0.6±0.5	0.7±0.3	—	—

Note: Lipid classes indicated are as follows: triglycerides (TG); wax esters (WE); cholesterol (CH); phospholipids (PL); and free fatty acids (FFA). Blank values indicate levels below detection (with no distinct peaks). Data are means ± SE.

0.3). We observed no differences in external morphology between sexes, except when gravid females were close to spawning. Our sample of 23 fish contained only one male, with final GSI of 29.7 % (Table 1).

The hepatosomatic index (HSI) indicated that liver mass decreased close to spawning (Fig. 1*b*), with most of the change occurring within the last 2 months. In ripe females, the HSI decreased from 5.4% in July–September to 1.2% in February, a drop of 78%, whereas in unripe females the decrease was less (to 1.5%). The single ripe male also had a much lower HSI at the time of spawning (0.7%), as compared with unripe males in July–September (4.9%) (Fig. 1*b*).

The somatosomatic index (SSI), suggested that muscle mass changed little until just prior to spawning (Fig. 1*c*). At spawning SSI declined by 5.0% in ripe females (*t*-test, $p = 0.0006$) and by 6.7% in the ripe male.

At the time of spawning, gonads of ripe females consisted of 92% water, whereas gonads of unripe females had less water (76.5%; Table 1). Ripe female gonads contained 4.1% protein and 1.2% lipids based on wet weight (51.0 and 15.2% based on dry weight). The mean energy content was 1.84 kJ/g wet weight (Table 1), or 23.92 kJ/g ash-free dry weight. Gonads from unripe females had significantly higher levels (*t*-test, $p = 0.0001$) of protein and lipids, 15.7 and 4.5% of respective wet weights (66.8 and 19.3% of respective dry weights), and thus higher energy content (25.63 kJ/g ash-free dry weight) than ripe females (*t*-test, $p = 0.0001$).

From August to December/January, female gonads increased significantly in both lipid and, especially, protein content (*t*-tests, $p < 0.05$; Table 2). Thus, the lipid to protein ratio of female gonads decreased from 0.38 in August to 0.27 in December/January as more protein was incorporated. This ratio changed little prior to spawning, at which time it was 0.29 for both ripe and unripe females.

Table 3. Potential fecundity estimated based on 1.0-g subsamples, containing 372 ± 21 (SE) eggs, taken from the middle of each gonad. Mean egg diameter (±SE) was determined from measurements of 22–25 fresh eggs.

Fish No.	Weight (g)	Gonad wt. (g)	GSI (%)	Fecundity (no. of eggs)	Egg diameter (mm)
1	201.4	97.7	48.5	32 101	1.65±0.04
2	141.6	66.9	47.2	24 692	1.67±0.04
3	123.2	62.0	50.3	25 234	1.66±0.02
4	149.4	77.5	51.9	27 358	1.48±0.04
5	156.5	71.5	45.7	23 205	1.56±0.03
6	164.5	78.9	48.0	32 822	1.49±0.03
7	127.0	64.8	51.0	20 913	1.80±0.02
8	117.8	60.0	50.9	21 360	1.68±0.05
9	160.9	82.6	51.3	26 914	1.77±0.03
10	151.1	72.0	47.7	33 251	1.68±0.04
11	166.8	73.7	44.2	23 289	1.70±0.02
Mean	150.9	73.4	48.8	26 467	1.65
SE	7.2	3.2	0.8	1353.4	0.03

Table 4. Mean energy loss (±SE) during gonadal development and spawning in Arctic cod.

Maturity/sex	*n*	TEG (kJ)	TEL (kJ)	TEC (kJ)	TED (kJ)	TEG/TED × 100 (%)
Ripe females	17	125.15±8.15	252.09±2.08	54.48±0.45	306.57±2.53	40.84±2.62
Unripe females	5	116.43±4.09	248.14±5.14	85.52±1.77	333.66±6.92	34.89±0.93
Ripe male	1	66.78	238.41	65.65	304.06	21.96

Note: Loss from gonads as spawning products (TEG) was determined from weight and energy content of gonads (Table 1). Losses from liver (TEL) and carcass muscle (TEC) were determined from decreases in hepatosomatic and somatosomatic indices, and the energy content of liver and carcass. TEG/TED is energy loss as spawning products relative to energy loss during gonadal development (TED = TEL + TEC).

A similar pattern was apparent in males, although the lipid to protein ratio was always lower than in females. The single ripe male had a lipid to protein ratio of 0.16.

Livers of females increased significantly in protein content (*t*-test, $p = 0.0002$), whereas for males it changed little (Table 2). In contrast lipid content in the liver decreased during gonad development, especially for females (*t*-test, $p = 0.0004$; Table 2). The relative amounts of specific lipid classes were different in gonads and livers (Table 2). Whereas livers contained mainly triglycerides (>90%), gonads contained little of this lipid class (<7%; Table 2). Gonads contained about 50% phospholipids during early development, and the amounts increased to >90% closer to spawning. During the early stages of development gonads also contained a high proportion of wax esters. In addition, gonads and livers contained low levels (<10%) of cholesterol and free fatty acids. There were no major differences in lipid class composition between males and females in either gonads or livers.

Fecundity and egg size

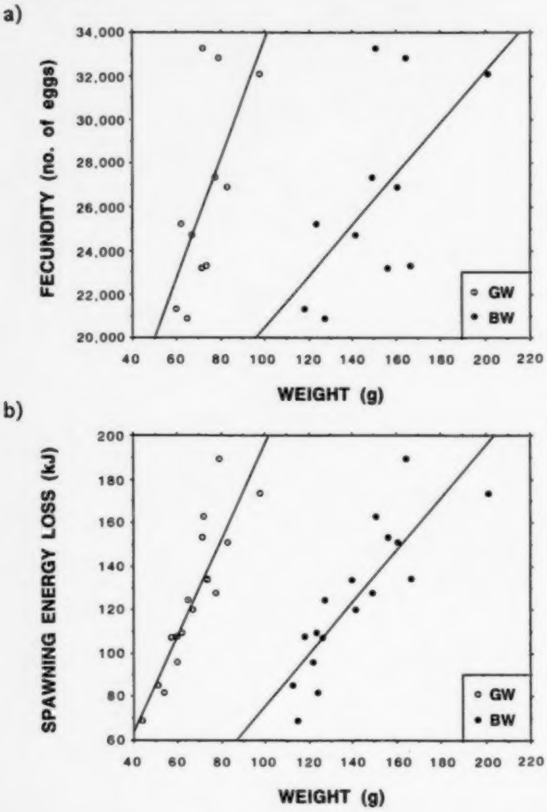
Potential fecundity was 26 470 ± 1350 (SE) eggs for fish of 151 ± 7 g wet weight (Table 3), which equaled 177 ± 7 (SE) eggs/g wet weight of fish. The mean egg diameter of stripped eggs, before water hardening, was 1.65 ± 0.03 (SE) mm. Ripe eggs were unpigmented, had a thin chorion, and could easily be seen through the ovarian wall. There was no significant relationship between the diameter of ripe eggs and gonad weight ($r^2 = 0.043$, $p = 0.542$) or potential fecundity ($r^2 = 0.156$, $p = 0.229$) (Table 3). Ripe gonad weight increased linearly with whole body weight ($r^2 = 0.871$, $p = 0.0001$), and fecundity increased with gonad weight or with whole body weight (Fig. 2*a*). Fecundity (FE) also increased with fork length (FL), although this relationship was not significant ($r^2 = 0.212$, $p = 0.154$).

Energy costs

Energy invested in gonads was substantial for Arctic cod. We expected that fish would die after spawning because of high energy costs, but several individuals that spawned naturally in the laboratory survived. Total energy invested in egg mass increased with increasing gonad size and whole body weight (Fig. 2*b*). The largest fish thus had the largest absolute energy loss because they had the highest reproductive output. The mean energy loss during spawning was 125 kJ per ripe female and 67 kJ for the ripe male (Table 4). Unripe fish had allocated almost as much energy into gonads (116 kJ) as ripe fish at the time of spawning (Table 4).

Energy was lost from liver and muscle (carcass) during the gonadal development period. This amounted to about

Fig. 2. Fecundity (FE) and spawning energy loss (SL) of ripe Arctic cod, stripped at the time of spawning (February), as a function of wet weights (g) of gonads (GW) and whole body (BW). (*a*) Potential fecundity, as number of eggs: $FE = 274.94 \cdot GW + 6281.68$; $r^2 = 0.433$, $p = 0.0278$; $FE = 116.81 \cdot BW + 8837.12$; $r^2 = 0.382$, $p = 0.0427$. (*b*) Potential spawning energy loss, as energy contents (kJ) of eggs: $SL = 2.265 \cdot GW - 27.694$, $r^2 = 0.781$, $p = 0.0001$; $SL = 1.203 \cdot BW - 44.606$, $r^2 = 0.728$, $p = 0.0001$.



300 kJ for both females and the male (Table 4), with liver being the primary energy source. Ripe females lost 87.5 and 16.0% of the initial energy in liver and muscle, respectively, whereas the ripe male lost 86.5 and 23.0% of the energy originally in these tissues. About 40% of the energy lost from liver and muscle was incorporated into gonads in ripe females, 35% in unripe females and 22% in the ripe male (Table 4), assuming little energy was obtained from other sources.

Sex steroids

Plasma levels of sex steroids [T], [E_2], and [11-KT], at the time of spawning, were significantly lower in ripe fish than in unripe fish (*t*-tests, $p = 0.0001$), although the ratio of [E_2]/[T] was about 50% higher in ripe versus unripe females (Table 5). In contrast, the ratio of [11-KT]/[T] was about 20% higher in unripe females. The single ripe male had steroid levels similar to the ripe females, although the ratio of [E_2] and [11-KT] to [T] was lower (Table 5).

Discussion

Arctic cod invest a large amount of energy in reproduction. Weight loss in spawning was 30–50% higher than that of Atlantic cod (*Gadus morhua* L.), Pacific cod (*Gadus macrocephalus* Tilesius, 1810), and walleye pollock (*Theragra chalcogramma* Pallas, 1814) (Eliassen and Vahl 1982; Smith et al. 1988, 1990). Arctic cod lost about 50% of body mass as eggs, and the energy for gonadal development was drawn from liver, predominantly, and muscle. Both mass and lipid content of the liver, and mass of muscle, decreased during gonadal growth. This is similar to what has been observed for spawning Atlantic cod, where females withdrew lipids from the liver and protein from somatic tissues (Dambergs 1964; Krivobok and Tokareva 1973; Kjesbu et al. 1991). Protein is assumed to be mobilized from the white muscle rather than the red muscle (Love 1960; Greer Walker 1971).

Our estimated energy loss from gonads may be slightly greater than actual loss during spawning, as some gonad mass may be retained after spawning; about 9% retention has been observed for Pacific and Atlantic cod (Smith et al. 1990). However, Atlantic cod are batch spawners, releasing portions of eggs as they develop, whereas eggs of Arctic cod seem to mature simultaneously, and females are thus capable of being complete or synchronous spawners (Graham and Hop 1995). If all eggs are fully developed, Arctic cod probably retain little gonad mass after spawning, although we could not measure this in stripped fish. In wild fish it has been observed that gonads of spawned-out fish regress to a state indistinguishable from gonads of immature cod (Sameoto 1984).

Energy loss from the liver was likely underestimated, because we had no estimate of the decrease in its energy content during the last month prior to spawning. There are no published estimates of energy loss in Arctic cod during gonadal development with which to compare our values. However, loss of energy from the liver was 71% for Pacific cod (Smith et al. 1990) and 67% for Atlantic cod (Eliassen and Vahl 1982). Our estimate of energy loss from livers in Arctic cod was about 15% higher, but then Arctic cod lost considerably more body mass than other gadids for which data are available (Eliassen and Vahl 1982; Smith et al. 1988, 1990).

Energy loss from somatic tissue of Arctic cod may also have been underestimated, because we did not account for the additional weight of stomach contents in samples near spawning. Stomach contents of unripe Arctic cod of 245 mm fork length (mean size of ripe females) may comprise up to 15% of body weight if fish are fed once to satiation (H. Hop, unpublished data), although long-term feeding to satiation gives substantially lower values. In a different experiment where Arctic cod ($n = 12$) were fed to satiation on capelin for 56 days (Hop 1994), the stomach content weight at the end of the experiment (mid-December) ranged from 0 to 4.3% of body weight (mean = 0.9%). In ripe fish, gonads occupy most of the body cavity so that stomach capacity is substantially reduced, and the weights of stomach content in

Table 5. Plasma levels of sex steroids in Arctic cod at the time of spawning (14 February 1992), with mean (±SE) hormone concentrations.

Maturity/sex	*n*	GSI (%)	[E_2] (pg/mL)	[11-KT] (pg/mL)	[T] (pg/mL)	[E_2]/[T] (%)	[11-KT]/[T] (%)
Ripe females	15	47.2±1.0	272±31	503±184	1210±278	32.6±3.8	30.3±5.1
Unripe females	5	22.3±0.9	831±192	2853±276	6092±784	15.6±4.7	50.6±9.7
Ripe male	1	29.7	193	235	1787	10.8	13.1

Note: Estradiol; E_2, 11-ketotestosterone, 11-KT; testosterone, T. Ratios (%) of [E_2] and [11-KT] to [T] are indicated. GSI is gonadosomatic index for fish sampled.

February were thus probably lower than the values in mid-December. We also did not account for loss in energy content of somatic tissue, although we did account for weight loss of this tissue. Our estimate of energy loss from somatic tissue relative to liver (18%) was still higher than for Atlantic cod (6%; Eliassen and Vahl 1982).

About 40% of the energy lost from liver and carcass was presumably incorporated into gonads of ripe females, with the other 60% being lost to biosynthetic processes and metabolism. An unknown amount of energy was obtained from ingested food, because Arctic cod continued to feed until the time of spawning. Ingested energy may be assimilated or lost in metabolism and as waste products (Brett and Groves 1979). The level of feeding was relatively low in our experiment, but if net energy were assimilated this could have reduced the energy loss from tissues and possibly enhanced GSI. In our study we could only account for the major losses associated with gonadal development and spawning, and we did not attempt to model a full energy budget for Arctic cod as in Hop (1994).

Potential fecundity of Arctic cod in our study was higher than what has generally been reported in the literature (e.g., Scott and Scott 1988), which are based on numbers from Russian waters (9000 – 21 000; Andriyashev 1954), although higher egg counts (30 000 – 67 000) were reported in one Russian study (Rass 1941). The lower fecundity reported by Andriyashev (1954) could have been the result of using smaller sized spawning fish. Even though our relationship between fork length and fecundity was not significant, it did indicate an increase in fecundity with length, as has been shown for Greenland cod (*Gadus ogac* Richardson, 1836) and Atlantic cod (Morin et al. 1991). Furthermore, an exponential relationship between fork length and gonad weight has been shown for Arctic cod (Lear 1980), and we found that fecundity increased with gonad weight. Andriyashev (1954) indicated that most Arctic cod in Russian waters mature at about 200 mm total length (FL = 192 mm) at 4 years of age, which would imply a fecundity of about 15 000 eggs (from our relationship) or 11 500 eggs (estimate based on Lear 1980 and Fig. 2*a*), which are within the range reported (9000 – 21 000). Our largest fish had a fecundity in the range reported by Rass (1941).

Egg diameter in our study was similar to what was reported by Andriyashev (1954) from Russian waters (1.6–1.8 mm). There was no significant relationship between egg size and fecundity in our study. Arctic cod eggs are larger and fewer as compared with other gadids (Andriyashev 1954), and it is evident that they vary little in size among regions. Egg diameter has been found to increase during August and September in Alaskan Arctic cod (Craig et al. 1982). The most rapid increase in egg diameter of Atlantic cod occurs just prior to ovulation when there is a massive influx of water into the oocytes (Kjesbu et al. 1992). The similar total energy content of gonads from ripe (125 kJ) and unripe (116 kJ) females indicated that the difference in GSI between these groups mainly resulted from water uptake at the time of spawning. It has been suggested that the mechanism involved is a breakdown of protein phosphate into free amino acids, which gives rise to an increase in osmolality and the influx of water into oocytes (Craik and Harvey 1984). The unripe females might have had a delayed influx of water and possibly could have spawned later in the breeding season.

Eggs of polar marine invertebrates are usually large and rich in lipids, a phenomenon known as Thorson's rule (Thorson 1950). There is also a general negative correlation between egg size and development rate in marine invertebrates, and it has been suggested that the slow rates of development for eggs of arctic invertebrates are the result of large egg size rather than low temperatures (Clarke 1983). This hypothesis could also apply to fishes, but it first needs to be verified experimentally with different sizes of eggs, from arctic and subarctic fish species, developing at low temperatures. Hatching of Arctic cod eggs under laboratory conditions occurs after 26–60 days (Aronovich et al. 1975; Graham and Hop 1995), although it has been estimated to occur after 45–90 days in the Barents Sea (Rass 1968). Atlantic cod eggs (1.2–1.6 mm) hatch after 50–60 days at surface temperatures of −1.5 to 0°C (Templeman 1981), which is similar to Arctic cod eggs at equivalent temperatures.

Even though the energy content of eggs from Arctic cod and Atlantic cod were similar (Hislop and Bell 1987), Arctic cod eggs contained less protein (51.0%) and relatively more lipids (15.2%) than Atlantic cod eggs (73–82% protein and 6–16% lipids; Lønning et al. 1988; Kjesbu et al. 1991). This suggests that the Arctic cod mobilizes less protein from muscle during gonadal development, likely because it has less muscle mass than the Atlantic cod. Ripe Arctic cod eggs contained much more water (92%) than ripe eggs of Atlantic cod (72%; Kjesbu et al. 1991), consistent with the latter containing more protein. On the other hand, Arctic cod do have a large liver from which lipids can be mobilized. A high amount of lipids in Arctic cod eggs may increase their buoyancy so that the eggs remain near the undersurface of the ice and possibly improves their resistance against freezing.

Lipids in the gonads and liver of Arctic cod differed, with mainly phospholipids in gonads and triglycerides in the liver. Phospholipids in gonads likely result from the breakdown of triglycerides (triacylglycerols) in the liver. Both phospholipids and triglycerides share a common precursor (diacylglycerol), and synthesis from this precursor involves two different enzymes to yield the final compounds (Lehninger 1982). This process demands energy that would account for some of the energy loss from liver and carcass. In polar fishes triglycerides are used for buoyancy (DeVries and Eastman 1978; Eastman and DeVries 1982), but a main function must also be energy storage as triglycerides are stored in Atlantic cod livers (Eliassen and Vahl 1982). In polar marine invertebrates the dominant storage lipid is always triglycerides, although Arctic copepods may also store wax esters and sterol esters at certain times of the year (Lee 1974; Clarke 1983). We detected only small amounts of wax esters in Arctic cod liver, although gonads contained relatively high amounts of wax esters and some cholesterol during early development. Ovarian lipids of many north-temperate fish are predominately phospholipids (Kaitaranta and Ackman 1981; Tocher and Sargent 1984). Both phospholipids and cholesterols are essential constituents of cell membranes (Geise 1966) and therefore important in formation of the chorion during egg development.

Sex steroid levels differed between ripe and unripe fish, with unripe fish having higher levels of each of the steroids investigated. The significance of lower steroid levels in ripe females is not known, but this is a common pattern that has also been observed in non-gadid species. For example, in both rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (Scott et al. 1980) and Atlantic halibut (*Hippoglossus hippoglossus* L.) (Methven et al. 1992), sex steroid levels increased during gonadal development and declined around the time of ovulation. In our experiment, ripe fish were sampled at a time when fish began spawning, and sex steroid levels had apparently already declined in most ripe fish. The high steroid levels, combined with relatively high GSI, in unripe fish, suggest that these fish could have ovulated at a later time the same season. The general spawning time for wild Arctic cod may extend over several months (Rass 1968).

More detailed studies are warranted to help clarify the endocrine changes associated with gonadal development and spawning in Arctic cod. It is known from other teleosts that increased sex steroid levels prior to the spawning period are important for the development of secondary sex characteristics (Fostier et al. 1983; Cardwell and Liley 1991), although no such changes were observed in Arctic cod. Other functions, which need to be further studied, include expression of sexual behaviors (Fostier et al. 1983; Liley and Stacey 1983) and hormonal control of ovulation (Peter 1983; Peter et al. 1991). The gonadal steroid estradiol-17β is the primary stimulator of liver vitellogenin production (Ng and Idler 1983) and increased estradiol levels prior to spawning are essential for increased production of yolk and chorionic proteins (Oppen-Berntsen et al. 1990), which subsequently enhances the development of embryos.

Initially, male gonads developed more rapidly than female gonads, reaching full size about 2 months before females. Differential rates of gonadal development between males and females have also been noted in other studies of this species (Lear 1979). This ensures that males are ready to spawn when females first start to ovulate. Ripe females likely cannot defer ovulation, and a slight timing mismatch in spawning readiness would lower the chance of successful fertilization. Our study indicated that the energy loss during spawning was less for the ripe male than for ripe females, although the energy loss from tissues during gonadal development was approximately the same for both sexes. Males probably spend extra energy maintaining ripe gonads for a longer time than females and may also spend more energy on spawning behavior.

Nikol'skii (1954) indicated that Arctic cod probably spawn only once during their life cycle. The energy cost of spawning is high, and spawning mortality in nature may therefore be substantial. Moskalenko (1964) described post-spawning Arctic cod as completely emaciated and noted cases where fish perished in large numbers after spawning. Arctic cod may compromise post-spawning survival with reproductive output, which would account for the low abundance of age-classes >4 years in samples of this species (Hop et al. 1995). Even though post-spawning survival may be compromised in nature, it has been observed, under laboratory conditions, that Arctic cod are repeat spawners that do not die after spawning (Graham and Hop 1995).

Post-spawning survival in Arctic cod may be enhanced by several factors, including low respiration energy costs (Hop and Graham 1995) and their ability to resume feeding immediately after spawning (Graham and Hop 1995). Field samples from Russian waters did contain post-spawners and indicated that fish fed both before (66.7%) and after spawning (43.5%) if food was available (Altukhov 1981). Arctic cod spawn under the ice at a time when most predators, except ringed seal (*Phoca hispida*), are absent (Davis et al. 1980), which also would reduce mortality of emaciated post-spawners.

These factors support repeated spawning of wild Arctic cod in spite of high amounts of energy allocated to the first reproduction. Repeated spawning for this species would likely enhance fitness, as the total reproductive output for an individual would be higher if the fish also spawned at a larger size when fecundity is greater. However, the chance of an individual surviving to the next spawning season may be rather low because of high predation rates on this species during the open-water season (Welch et al. 1992; Welch et al. 1993).

The reproductive strategy for Arctic cod therefore seems to be a delicate balance between reproductive costs and post-spawning survival. It may have evolved as an adaptive life-history strategy for a fish species that has slow growth rates, but early maturation and short life span, as well as high predation rates in Arctic waters. A high energy investment in the first reproduction must be a successful strategy for Arctic cod, given the probably very high abundance of this species in the Canadian high Arctic (Welch et al. 1992).

Our laboratory study has indicated energy allocation and costs during gonadal development and spawning but could only give limited answers without a considerable field component. Thus, further studies are clearly needed to address the balance between reproductive costs and post-spawning survival.

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Plankton and water chemistry in Lake Njupfatet before and after liming

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Abstract: Moderately acidified Lake Njupfatet was studied during 2 consecutive years, before and after liming (calcite), and compared with corresponding data from six reference lakes. After liming, the concentration of total phosphorus in the lake water decreased by some 30% as did the concentrations of particulate carbon, particulate nitrogen, particulate phosphorus, and phytoplankton biomass. Because of significant increases of inorganic nitrogen and dissolved organic carbon, the concentrations of total nitrogen and total organic carbon remained unchanged after liming. Before liming, there was a close balance between phosphorus and nitrogen limitation of phytoplankton growth, but we conclude that after liming the reduced concentrations of phosphorus induced phosphorus limitation of phytoplankton growth. Liming changed the phytoplankton community structure, most importantly causing the complete loss of the dominant species before liming, the cyanophyte *Merismopedia tenuissima*. The decrease in total biomass of phytoplankton was not compensated for by a corresponding increase in other species. After liming total biomasses of bacterioplankton and protozoan zooplankton did not change, while total biomass of metazoan zooplankton increased; hence, total plankton community carbon remained unchanged. Phytoplankton, protozoan, and metazoan zooplankton diversity (Shannon index) did not change after liming. Zooplankton biomass remained heavily dominated by calanoid copepods, typical of acidified lakes.

Résumé : Nous avons étudié pendant 2 années consécutives le lac Njupfatet, qui est légèrement acidifié, avant et après chaulage (avec de la calcite), et nous avons comparé les données obtenues à celles de six lacs témoins. Après le chaulage, la concentration de phosphore total dans l'eau du lac a baissé d'environ 30%, tout comme les concentrations de carbone particulaire, d'azote particulaire et de phosphore particulaire, ainsi que la biomasse phytoplanctonique. À cause des réductions significatives de l'azote inorganique et du carbone organique dissous, les concentrations d'azote total et de carbone organique total sont demeurées les mêmes après le chaulage. Avant le chaulage, il existait un équilibre étroit entre le phosphore et la limitation de la croissance phytoplanctonique par l'azote, mais nous concluons qu'après le chaulage la réduction de la concentration de phosphore a induit une limitation de la croissance phytoplanctonique par le phosphore. Le chaulage a modifié la structure de la communauté phytoplanctonique, et son effet le plus important a été la disparition totale de l'espèce qui dominait avant le chaulage, le cyanophyte *Merismopedia tenuissima*. La baisse de la biomasse totale de phytoplancton n'a pas été compensée par une croissance correspondante chez d'autres espèces. Après le chaulage, les biomasses totales du bactérioplancton et des protozoaires zooplanctoniques n'ont pas changé, tandis que la biomasse totale des métazoaires zooplanctoniques a augmenté; le carbone total de la communauté planctonique est donc demeuré inchangé. La diversité du phytoplancton et des protozoaires et métazoaires du zooplancton (indice de Shannon) n'a pas changé après le chaulage. La biomasse zooplanctonique est restée fortement dominée par les copépodes calanoïdes, ce qui est caractéristique des lacs acidifiés.

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Introduction

Sweden, more than any other country, has striven to counteract the negative effects of acidification by liming. The governmental grant-financed liming program started in 1977, and by 1990 some 6000 lakes and several hundred running waters had been treated (Bernes 1991). Modern official guidelines state that the aim is to ensure the survival of natural fauna and flora and to allow organisms to recolonize damaged areas.

Whether liming really restores the original status of the ecosystem or instead contributes to the formation of new ecosystems is difficult to judge, as preacidification data are usually lacking. Studies on effects have concentrated on water chemistry and the structure and reproduction of fish populations. Other biota have received less attention especially as regards quantitative relationships, productivity, and trophic level interactions.

Phosphorus is generally believed to be the primary limiting factor for phytoplankton production in oligotrophic lakes (Schindler 1977) and, in this respect, it has also been identified as a key element in acidified waters (Grahn et al. 1974). Data from Lake Gårdsjön (Broberg and Persson 1984; Persson and Broberg 1985) indicate reduced transport of phosphorus to acidified lakes due to trapping of phosphorus in the drainage area. If this is true, liming of the lake water alone or complemented with liming of the inflowing water will not restore the original conditions. Furthermore, additions of lime may lead to precipitation of existing lake water phosphorus, resulting in further oligotrophication of the system. However, data on liming effects on phosphorus are highly divergent. Increasing concentrations have been reported from several Swedish west-coast lakes but in those cases samples have been taken only from 0.5 m depth (Appelberg et al. 1990, Hörnström et al. 1993) and the result may instead reflect vertical redistributions of biota. That liming leads to decreasing concentrations of phosphorus has been suggested by Olofsson et al. (1988), who evaluated regional lake data, and was found by Molot et al. 1990*a*). Several studies show minor or no changes (Broberg 1988 and references therein).

Tests on limiting nutrients have revealed a close balance between phosphorus and nitrogen limitation of phytoplankton production in acidified (Blomqvist et al. 1989) as well as other lakes (Elser et al. 1990). Recent studies also indicate a close balance between carbon, phosphorus, and nitrogen limitation of bacterioplankton production (Bell et al. 1993). Thus, when evaluating effects of lime treatment on biota it is necessary to take into consideration changes also in nutrients other than phosphorus. With regards to the effects of liming on dissolved organic carbon and nitrogen, however, the results are as contradictory as those for phosphorus (reviews by Broberg 1987, 1988).

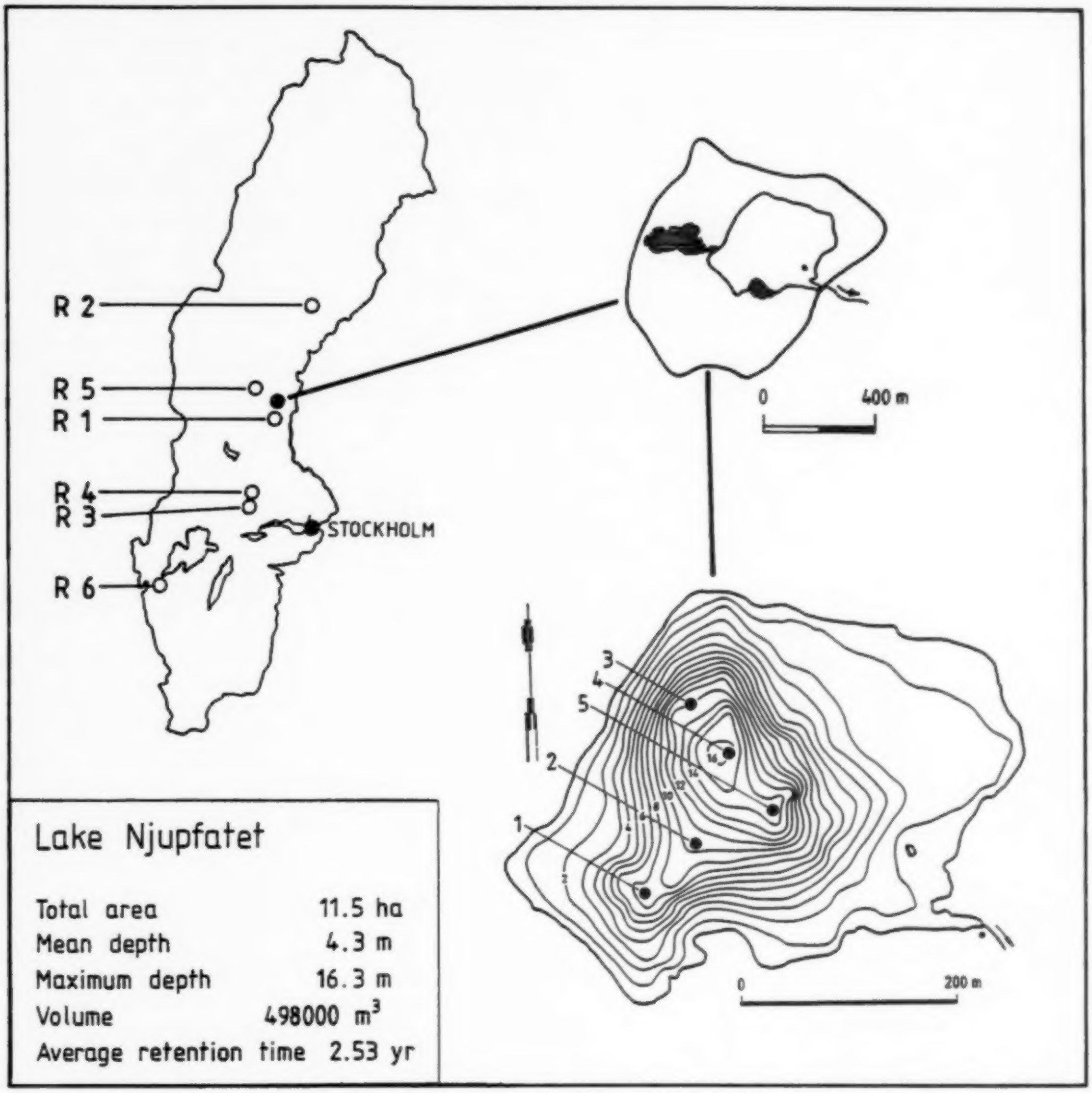
In accordance with the results regarding phosphorus, but often without indications of a direct coupling between the two parameters, phytoplankton have been reported to respond to liming by increased (Larsson 1988, Hörnström et al. 1993), decreased (Olofsson et al. 1988, Hörnström et al. 1993), or unaltered (Molot et al. 1990*b*, Hörnström et al. 1993) biomass. Phytoplankton species diversity usually increases after liming (Eriksson et al. 1983; Stokes 1986; Larsson 1988), often in combination with considerable changes in community structure. The most conspicuous feature of phytoplankton in acidified lakes is a late summer dominance of dinoflagellates or cyanophytes (Blomqvist et al. 1989). Liming appears to alter this structure towards increased amounts of chrysophytes, cryptophytes, and green algae both in the short (Bukaveckas 1989) and long term perspectives (e.g., Brettum and Hindar 1985; Molot et al. 1990*b*).

Whether liming can restore the zooplankton community to preacidification conditions is also unclear as the results are contradictory. Metazoan zooplankton communities respond to acidification by reduced species diversity and by considerable structural changes (Geelen and Leuven 1986). All major metazoan groups are affected but daphnids are known to be extremely sensitive (Nilssen 1980; Stensson et al. 1993). However, Geelen and Leuven (1986) in their extensive review did not find any evidence that acidification reduced zooplankton biomass. Liming, on the other hand, was found to increase (e.g., Nyberg 1984; Raddum et al. 1986; Hörnström et al. 1993), decrease (e.g., Yan and Dillon 1984), or have no influence at all (e.g., Stensson and Oscarsson 1985; Hörnström et al. 1993) on zooplankton biomass. Stensson et al. (1993) conclude that "the simple acid-state crustacean zooplankton community, with low biomass and dominance of calanoids may continue some years after liming." The observed changes in zooplankton community structure because of acidification may have been caused by several factors: direct physiological stress because of hydrogen and metal ions, altered food concentrations because of dominance shifts in phytoplankton communities, and shifts in the structure of fish and invertebrate planktivore communities. The structural changes in zooplankton after liming appear to be highly dependent on the degree of damage to the entire ecosystem before liming. For example, in lakes were the fish populations have been eliminated, the major structural changes after liming do not occur until the lakes have been restocked with fish (e.g., Nyberg 1984; Henrikson et al. 1985).

Few data exist on liming effects on bacterioplankton biomass and production, mixo- and hetero-trophic nanoflagellates and protozoan zooplankton, i.e., the pelagic food chain often termed "the microbial loop." Beaver and Crisman (1981) found that small ciliated protozoa were disfavoured in acidified lakes, probably as a result of decreased food availability. They referred to the findings of Bick and Drews (1973) and Andersson et al. (1974) stating that both bacterial biomass and activity decline with increasing acidity. Increased bacterial activity and (or) biomass after liming has usually been found (e.g., Gahnström 1988; Salonen et al. 1990 and references therein) although exceptions exist (Salonen et al. 1990).

Thus, the pelagic communities seldom show any clear-cut responses to liming. This is not especially surprising as the organisms are linked by complex trophic interactions and depending on both bottom-up and top-down forces that may magnify as well as diminish the effect of liming. The aim of the integrated "Lake Njupfatet project" is to study combined bottom-up and top-down effects on pelagic trophic interactions in an acidified and limed lake by means of controlled perturbations in small-scale in situ enclosures. To be able to relate the results of the enclosure studies to the conditions in the lake, the pelagic ecosystem was monitored

Fig. 1. The location of L. Njupfatet and the reference lakes (R1–R6), the drainage area of L. Njupfatet and the location of the limed bogs (hatched area), a bathymetric map of L. Njupfatet showing the location of the sampling stations (1–5), lake morphometry, volume, and average retention time of the water.


R 2
R 5
R 1
R 4
R 3
STOCKHOLM
R 6
0
400 m
3
4
5
2
1
16
14
12
10
8
6
4
2
0
200 m
Lake Njupfatet
Total area 11.5 ha
Mean depth 4.3 m
Maximum depth 16.3 m
Volume 498000 m³
Average retention time 2.53 yr

during the 2 years, one prior to and one after liming. This paper reports quantitative relationships between different compartments of the pelagic ecosystem in the lake before and after lime treatment, and compares the changes occurring within L. Njupfatet after liming to the situation in six unlimed National Swedish reference lakes.

Study site and reference lakes

Lake Njupfatet (Swedish lake number 687837-153009) is a small (area 11.5 ha), oligotrophic clearwater lake located in central Sweden at 62°01′N, 16°23′E (Fig. 1). It is a headwater lake with a total drainage are of 62.5 ha, of which the lake constitutes 18%. Geologically, the area is dominated by acidic granites covered by morainic deposits and it has an altitude of 426–368 m above sea level. The vegetation principally consists of mature coniferous forest, dominated by *Pinus silvestris* and *Picea abies*. Some small *Sphagnum* bogs are located in the vicinity of the lake. Apart from air-borne pollutants, the influence by human activities is very low. Annual mean temperature in the area is +4°C and annual precipitation is 585 mm (SMHI).

The macrophyte vegetation in the lake is sparse, dominated by *Nymphaea alba*. No benthic mats of *Sphagnum* or other mosses were observed. In shallow areas, zygnematales and red algae (*Batrachospermum*) were fairly abundant. Two species of fish were present in the lake in 1985, crucian carp (*Carassius carassius* L.) and arctic char (*Salvelinus alpinus* L.). In 1989 only crucian carp was caught. The populations during both surveys consisted of large, presumably nonreproducing, specimens. If reproduction occurred, there were at least no signs of survival of juveniles.

As reference material for evaluation of normal year-to-year variations, data from six National Swedish liming reference lakes were used. The selected lakes (Fig. 1) were those located as close to L. Njupfatet as possible within the forested, moderate altitude region: Lake 1, Stensjön (61°38′N, 16°34′E, 70 ha, max. depth 8.5 m); Lake 2, Remmarsjön (63°51′N, 18°16′E, 135 ha, max. depth 14 m); Lake 3, Övre Skärsjön (59°50′N, 15°33′E, 165 ha, max. depth 32 m); Lake 4, Mäsen (60°01′N, 15°39′E, 37 ha, max. depth 24 m); Lake 5, Täringen (62°15′N, 15°42′E, 174 ha, max. depth 19 m); Lake 6, Härsvattnet (58°01′N, 12°01′E, 19 ha, max. depth 26 m). These data, which are available from the National Swedish Environmental Protection Board, represent seven samplings per lake and year (February and then monthly during the open water season, May–October) in all lakes but southernmost Lake Härsvattnet, in which eight samplings (February and then monthly, April–October) were performed per year. In each lake a depth profile of samples are taken, at 0.5, 2 (sometimes excluded), 5, 10 m depth, and also 1 m above the bottom. The depth of the latter sample may vary according to which depth was found during a particular sampling event. This reference data material is limited compared with that from L. Njupfatet with regards to the number of parameters analysed and includes only pH, conductivity, water colour (absorbancy at 420 nm), total nitrogen, ammonium nitrogen, nitrate nitrogen, total phosphorus, soluble reactive phosphorus, and phytoplankton biomass (microscopic counting). For a more thorough description of the aim of this reference lake material, the sampling, and analysis program see Willén et al. (1990).

Methods

A total of 26 samplings were performed between November 1988 and October 1990 in Lake Njupfatet, 13 samplings before and 13 samplings after liming. Each year, three samplings were carried out from ice with 2-months intervals. The remaining samplings were carried out biweekly from May to October. Water temperature was measured at one meter intervals from surface to bottom using the thermometer inside the Ruttner sampler. Samples for analysis of dissolved oxygen (O_2) were taken from 0.5, 5, 10, and 15 m. Volume-weighted samples representing 0–6 and 6–12 m were taken with a 2 m long tube sampler for analysis of water chemistry and plankton. Samplings were performed at five stations randomly distributed in the central part of the lake where the water depth exceeded 8 m (Fig. 1). Samples representing 0–2, 2–4, and 4–6 m, were mixed in a bucket in the proportions 5:3:2 (one tube representing 0–2 m taken at each station; one tube representing 2–4 m taken at stations 1, 4, and 5; and one tube representing 4–6 m taken at stations 1 and 4. The same proportion (5:3:2) was used for 6–8 (all stations), 8–10 (stations 2, 4, and 5), and 10–12 m (stations 4 and 5), in both cases corresponding to the proportion by volume between these layers in the lake. Subsamples representing 0–6 and 6–12 m were then taken from the buckets in the following order: 2 L of water for analysis of water chemistry and bacterioplankton abundance, and 100 mL of water for analysis of phytoplankton and protozoa. The remaining volume (18 L) was filtered through a 40-μm net and the animals retained (minimum number found in a sample 1869) were transferred to 100-mL bottles for analysis of metazoan zooplankton. Phyto- and zoo-plankton samples were immediately preserved with acidified Lugol's solution. Bacterioplankton samples (20 mL) were preserved with formaldehyde (final conc. 4%).

The water was then transported to the laboratory where pH, alkalinity, conductivity, soluble reactive phosphorus (SRP), ammonium nitrogen (NH_4-N), and absorbancy at 420 nm wavelength were measured within 24 h. When no other reference is given, the analyses were carried out according to Swedish standards (SIS 1992). SRP was analysed according to Murphy and Riley (1962) after filtration through a Gelman 0.45-μm membrane filter. NH_4-N was analysed according to Chaney and Marbach (1962). Absorbancy at 420 nm wavelength was measured after filtration through a Whatman GF/F-filter. Suspended matter (Susp.) was measured by filtering water through a preweighed Whatman GF/C-filter, letting it dry in room temperature overnight, and weighing it again. The particles retained by the Whatman GF/C-filter were then analysed for their content of carbon (PC) and nitrogen (PN) using dry ignition and IR detection (Carlo Erba Elemental Analyser 1106). The remaining water was preserved with $HgCl_2$ and stored at 4°C until analysis. Total phosphorus (TP) was analysed by persulfate digestion and photometric determination of phosphomolybdate (Menzel and Corwin 1965). Particulate phosphorus (PP) was analysed on the particles retained by a Gelman 0.45-μm membrane filter by photometric determination of phosphomolybdate, after digestion in a boiling mixture of acids (Ahlgren and Ahlgren 1976). Dissolved organic phosphorus (DOP) was calculated as TP minus PP minus SRP. Nitrate (NO_3-N) and nitrite (NO_2-N) were analysed according to Wood et al. (1967) and Kjeldahl nitrogen (Kj-N) according to Jönsson (1966). Total nitrogen (TN) was calculated as the sum of Kj-N, NO_3-N, and NO_2-N. Total inorganic nitrogen (TIN) was calculated as the sum of NH_4-N, NO_3-N, and NO_2-N. Dissolved organic nitrogen (DON) was calculated as Kj-N minus PN minus NH_4-N. Dissolved organic carbon (DOC) was analysed by dry combustion and IR detection of formed carbon dioxide using a Shimadsu carbon analyser. Total organic carbon (TOC) was calculated as the sum of PC and DOC. Dissolved oxygen (O_2) was analysed by Winkler titration.

Phytoplankton and protozoan zooplankton were identified and counted with an inverted microscope after sedimentation of 10 and 25 mL of water, respectively. Biovolumes were estimated using geometrical formulas and converted to biomass assuming a density of 1 g/cm^3. Metazoan

Table 1. Mean values ± standard deviations (February and May–October) and the relative change (the difference between the years as a percentage of the 1st year) of some water chemistry parameters and total phytoplankton biomass in six Swedish liming reference lakes (1–6, lake names and locations see Study sites) and in Lake Njupfatet (Njup.).

Parameter/year	Lake 1	Lake 2	Lake 3	Lake 4	Lake 5	Lake 6	Njup.
pH (units)							
1989	6.2±0.2	6.1±0.1	5.3±0.1	6.5±0.1	6.5±0.2	4.4±0.0	5.7±0.2
1990	6.2±0.1	6.1±0.1	5.2±0.1	6.4±0.1	6.4±0.1	4.4±0.1	7.2±0.3
Change (%)	−0.4	−0.5	1.5	−0.9	−1.1	−0.7	97
Conductivity (mS/m)							
1989	1.9±0.1	2.1±0.3	3.3±0.1	5.1±0.2	2.7±0.1	7.2±0.2	1.9±0.1
1990	1.9±0.1	2.1±0.3	3.4±0.0	5.1±0.1	2.9±0.1	7.9±0.1	4.2±0.1
Change (%)	−0.5	0.5	4.2	0.6	6.3	9	121
Absorbancy (420 nm)[a]							
1989	74±16	155±16	110±21	59±8	75±16	8±4	2±1
1990	72±20	149±39	80±8	47±6	65±8	9±6	3±1
Change (%)	−2.3	−3.9	−27	−20	13	13	50
TN (μg/L)							
1989	241±43	281±77	405±41	352±36	294±100	376±44	231±49
1990	251±56	308±59	392±30	349±81	275±24	343±41	270±38
Change (%)	4	9.6	−3.2	−0.9	−6.5	−8	17
NH_4-N (μg/L)							
1989	16±10	12±5	19±4	30±24	16±13	54±12	15±17
1990	12±6	18±8	17±9	30±22	14±5	64±10	15±10
Change (%)	−25	50	−11	0	−13	−18	0
NO_3-N (μg/L)							
1989	16±13	22±18	212±19	27±25	22±13	185±22	9±9
1990	13±8	11±12	205±13	38±19	18±8	189±21	26±23
Change (%)	−19	−50	−3.3	41	−18	2	188
TP (μg/L)							
1989	11±3	15±3	9±3	23±8	11±4	7±1	9±2
1990	10±2	11±2	8±1	16±8	15±14	8±3	7±1
Change (%)	−9	−26	−11	−30	36	14	−22
SRP (μg/L)							
1989	2.1±0.7	2.7±0.8	2.4±1.6	3.6±1.5	1.8±0.8	1.6±0.4	0.6±0.9
1990	2.4±0.5	2.8±1.2	1.8±0.7	3.7±2.1	5.3±7.8	1.7±0.6	1.1±0.7
Change (%)	14	3.7	−25	2.7	66	6	83
Total phytoplankton biomass (μg/L w.w.)							
1989	99±63	160±75	152±106	330±201	97±77	72±39	355±182
1990	443±156	196±97	73±48	270±124	64±13	166±174	238±128
Change (%)	347	23	−52	−18	−34	131	−33

[a] Values multiplied by 1000.

zooplankton samples were counted in their entirety with an inverted microscope in terms of number of animals and eggs. Biomasses were obtained using data on body length – dry weight relationships from other Swedish lakes (H. Olofsson, unpublished). Bacteria were counted and measured using acridine-orange staining and epifluorescence microscopy (Bell et al. 1983). Biomasses were converted to carbon using the following assumptions. Phytoplankton carbon: cyanophytes = 22% of the wet weight (Ahlgren 1983), chlorophytes = 16% of the wet weight and other algae = 11% of the wet weight (Lundgren 1978 and references), protozoan zooplankton carbon = 19% of the wet weight (Putt and Stoecker 1989), metazoan zooplankton carbon = 48% of the dry weight (Andersen and Hessen 1991). Bacterioplankton carbon was obtained by taking the mean cell volume of all cells (0.06 μm^3) and using a carbon:volume conversion factor of 0.282 pg C/μm^3 (Simon and Azam 1989).

Liming of the lake was performed in 1989 at the end of the autumn circulation period (November) according to standard procedures for Swedish lakes (Bernes 1991). Based on the average retention time of the water, the acid-neutralizing capacity was calculated to last for at least 5 years. Liming was administered by the Gävleborg County administrative board and sponsored by governmental grants to 100%. A total of 16 tonnes of finely ground limestone (calcite, particle size 0–0.2 mm) was spread over the lake by helicopter. Another 4 tonnes of lime was spread over

Table 2. Summary of two-way ANOVA, testing the combined and interacting effects of time and liming on the difference between years of different water chemistry variables and total phytoplankton biomass in the combined L. Njupfatet and reference lake material. Liming*month = interacting effect.

Response factor	*F*	*p*
Δ pH		
Liming	6.41	0.0156 *
Month	0.52	0.8346 ns
Liming*month	0.40	0.8445 ns
Δ Conductivity		
Liming	305	<0.0001 ***
Month	0.32	0.9547 ns
Liming*month	0.10	0.4230 ns
Δ Absorbancy		
Liming	1.62	0.2117 ns
Month	2.66	0.0225 *
Liming*month	0.34	0.8472 ns
Δ TN		
Liming	4.15	0.0487 *
Month	2.72	0.0180 *
Liming*month	0.29	0.9182 ns
Δ NH_4-N		
Liming	0.03	0.8720 ns
Month	0.81	0.5957 ns
Liming*month	1.92	0.1133 ns
Δ NO_3-N		
Liming	10.24	0.0028 **
Month	1.43	0.5957 ns
Liming*month	0.67	0.1133 ns
Δ TP		
Liming	0.27	0.6081 ns
Month	0.90	0.5300 ns
Liming*month	2.49	0.0483 *
Δ SRP		
Liming	0.26	0.6143 ns
Month	0.49	0.8579 ns
Liming*month	0.90	0.4896 ns
Δ Phytoplankton biomass		
Liming	3.45	0.0726 ns
Month	0.25	0.9686 ns
Liming*month	1.20	0.3299 ns

Note: *F*, *F* score; *p*, probability; ns, not significant. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

water outflows (into the lake) from two small *Sphagnum* bogs in S and W (Fig. 1). In total, the addition of calcite corresponded to 1.7 tonnes/ha.

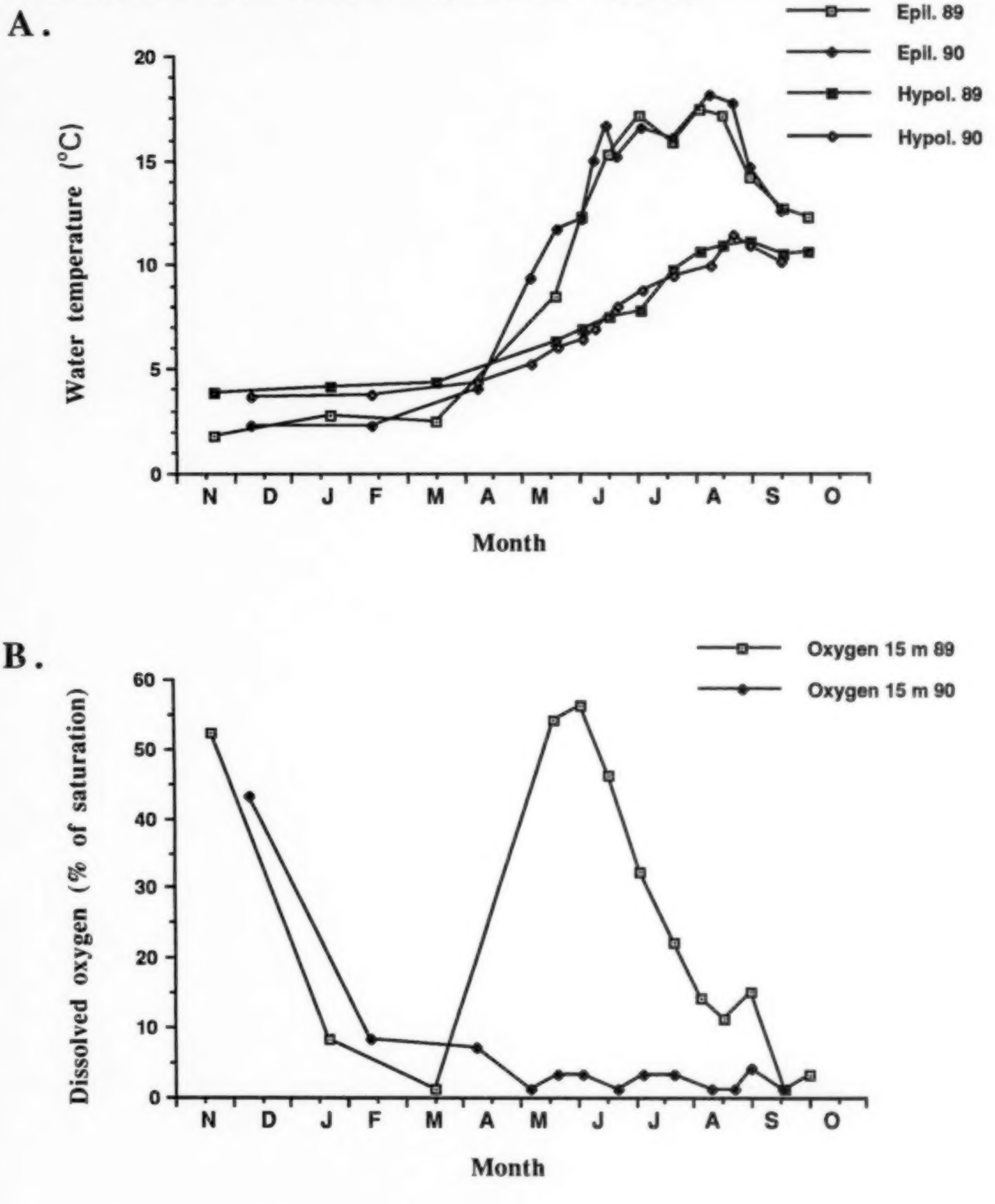
Statistical evaluation of differences between the 2 years on the entire data set (all parameters) from Lake Njupfatet was performed using Wilcoxon's signed rank test (Campbell 1974) on lake volume weighted data representing 0–12 m (97.3% of the lake volume). Statistical evaluation of those changes in Lake Njupfatet that could not be explained by natural year-to-year variations (as indicated by similar changes not occuring in the reference lake material) was obtained by (*i*) selecting only data from Lake Njupfatet from those months during which sampling was performed in the reference lakes (February and May–October) and (*ii*) computing the difference between years (1989–1990) for all parameters covered by both the reference material and L. Njupfatet and at all paired samplings within each locality, and (*iii*) evaluating the statistical significance of the changes in Lake Njupfatet relative to the changes in the reference lake material using two-way ANOVA with factors liming (unlimed–limed), time (month), and their term of interaction. Effects of liming were considered significant at a level $p < 0.05$.

Results

A comparison of means and variances of water chemistry parameters and total phytoplankton biomass in unlimed L. Njupfatet (1989) and in the reference lakes shows that L. Njupfatet in most aspects fitted well into the framework of the reference lakes. As regards pH, conductivity, NH_4-N, and TP, the data from L. Njupfatet clearly fell within the gradient formed by the reference lake data (Table 1). In relation to water colour (absorbancy at 420 nm), TN, NO_3-N, and total phytoplankton biomass the data from L. Njupfatet fell just outside the range of the reference data set, being clearer, lower in TN and in NO_3-N, and having a higher standing stock of phytoplankton than the reference lakes, but still within the ±10% of range that justifies extrapolation. The only parameter by which the data from L. Njupfatet deviated from those of the reference lakes by more than 10% was SRP. The concentrations of SRP in L. Njupfatet were lower than the reference lakes. According to the comparison of the changes in L. Njupfatet before and after liming and the difference between corresponding years in the reference lake material (Table 2), there was a significant positive (Table 1) effect of liming on pH, conductivity, and TN and a significant negative effect of liming in combination with time on TP. The decrease in total phytoplankton biomass in L. Njupfatet after liming was close to significant ($p < 0.075$), whereas liming did not lead to any significant changes in water colour, NH_4-N, or SRP.

Meteorologically in the L. Njupfatet area the 2 years were similar, with annual mean temperatures of 5.7 and 5.6°C, respectively, but were considerably warmer than normal (4°C). Annual precipitation was also similar, deviating from the long term average by −8 and +17%, respectively. The similarity between years is also evident from the small differences in water temperature in epilimnion and meta + hypolimnion (Fig. 2A). A slight but important difference, however, was the more rapid warming of the surface water immediately after icebreak in late April 1990, which resulted in the early formation of a thermocline and incomplete aeration of deeper strata during this year. At 0.5 and 5 m depth there were no significant differences in O_2 saturation between the years. At 10 m depth, the average O_2 saturation was some 50% lower in 1990 than in 1989 (averages 35 and 77% during the ice-free season) and at 15 m depth no oxygenation of the water took place at all in spring 1990 compared to an increase to over 50% saturation during the corresponding period in 1989

Fig. 2. (A) The average temperature in the epilimnetic and meta + hypolimnetic water. (B) The concentrations of dissolved oxygen at 15 m depth in L. Njupfatet during 1989 and 1990, respectively.



(Fig. 2B). As no increase in O_2 concentrations occurred at 10 or 15 m depths in spring 1990, the significant ($p < 0.05$ and 0.01, respectively) differences in O_2 saturation of the bottom water between the years should not be attributed to the calcite treatment but instead to incomplete circulation that may occur in some years in small, deep, oligotrophic lakes protected from winds.

The slightly acidic, oligotrophic clearwater character of L. Njupfatet before liming is well illustrated by the low averages of most water chemistry variables (Table 3). After

Table 3. Water chemistry in Lake Njupfatet before and after liming. Volume weighted values representing the 0–12 m water column.

	Before liming (1989)				After liming (1990)				
Parameter	*N*	Mean	SD	Range	*N*	Mean	SD	Range	% change
pH (units)	13	5.64	0.19	5.3–6.0	13	7.17	0.25	6.6–7.6	+97 ***
Conductivity (mS/m)	10	1.97	0.28	1.70–2.65	10	4.09	0.42	2.97–4.48	+108 ***
Alkalinity (mequiv./L)	10	0.03	0.01	0.01–0.05	10	0.28	0.05	0.19–0.34	+833 ***
Abs. 420 nm[a]	10	2.11	1.06	1.00–4.23	10	2.69	1.05	1.00–4.00	+27
Susp. (mg/L)	10	1.11	0.36	0.61–1.79	10	0.69	0.39	0.29–1.42	−38
TN (μg N/L)	13	242	53	171–301	13	260	41	206–349	+7
PN	13	64	19	39–107	13	45	14	15–66	−30 **
DON	13	149	45	75–215	13	171	37	122–252	+15
NO_3-N	13	11	11	3–34	13	25	21	10–78	+127 **
NO_2-N	13			1–2[b]	13			0–2	
NH_4-N	13	18	17	0–50	13	19	13	6–46	+6
TIN	13	30	24	7–83	13	45	29	19–109	+50 ***
TP (μg P/L)	13	9.3	2.0	6–13	13	6.6	1.4	4–9	−29 ***
PP	13	5.8	1.2	4–9	13	4.1	1.3	2–7	−29 ***
DOP	13	2.8	1.8	0–6	13	1.6	1.6	0–5	−43
SRP	13	0.7	1.0	0–3	13	1.0	0.7	0–2	+42
TOC (μg C/L)	13	3360	357	2790–4070	13	3539	435	2910–4430	+5
PC	13	596	118	350–779	13	426	123	265–699	−29 ***
DOC	13	2760	310	2390–3340	13	3110	490	2540–4170	+13 ***
TOC/TP	13	380	109	293–653	13	562	155	313–896	+48 ***
TN/TP	13	27	9	19–48	13	40	7	28–56	+48 ***
PC/PN	13	10	2	6–16	13	10	3	8–17	±0
PC/PP	13	107	31	41–158	13	109	32	51–161	+2
PN/PP	13	12	5	5–22	13	12	4	5–17	±0

Note: Abbreviations are explained in Methods. **, Wilcoxon's signed rank test $p < 0.05$; ***, Wilcoxon's signed rank test $p < 0.01$.

[a]The values have been multiplied with 1000.

[b]= close to or below the detection level of the method.

liming, pH, alkalinity, and conductivity of the water showed significant increases, with the two former parameters reaching values well above the pH of 6 and alkalinity of 0,1 mequiv./L that are set as chemical goals of a standard Swedish lake time treatment (Bernes 1991). No significant changes in water colour, measured as absorbancy at 420 nm, or suspended matter occurred after liming (Table 3).

NO_3-N increased after liming by more than 100%, while the other inorganic nutrient fractions remained unchanged. SRP and NO_2-N were usually close to or below the detection level of the methods applied (approx. 1 μg/L) which makes statistical evaluation uncertain. As a consequence of the increase in NO_3-N, TIN showed a significant increase after liming. Also DOC showed a slight but significant increase after liming.

TP and the particulate fractions of carbon, nitrogen and phosphorus decreased significantly after liming by the same order of magnitude (30%, Table 3). The decrease in concentrations of PC, PN, and TP expressed as the difference between the years was most pronounced during the winter months (sampling 1–3), especially PN and TP (Figs. 3A–3C). TN, TOC, and the dissolved organic fractions of nitrogen and phosphorus did not change significantly after liming (Table 3). As a result the TN:TP and TOC:TP ratios increased significantly after liming. The C:N, C:P, and N:P ratios in the particulate matter did not change after liming. The C:N:P ratio was 107:12:1 and 109:12:1 before and after liming, respectively.

Total phytoplankton biomass decreased significantly after liming (Table 4) by the same order of magnitude (30%) as the decreases in TP and particulate fractions of carbon, phosphorus, and nitrogen. Species diversity, both when calculated from number of individuals and from biomass, did not differ significantly between years. Of the 22 most important contributors to phytoplankton biomass, five taxa decreased significantly after liming, four taxa increased significantly, four new species not previously recorded appeared, while nine taxa did not change significantly. The most dramatic change in phytoplankton community composition was the total disappearance of the species dominating in the lake before liming, *Merismopedia tenuissima* Lemm (Cyanophyceae), of which not a single colony was found in the lake after liming. Other important species that decreased after liming were *Mallomonas fastigata* (Chrysophyceae) and *Gymnodinium uberrimum* (Dinophyceae). Among Cryptophyceae, all important species (*Cryptomonas*, *Rhodomonas*, and *Katablepharis*) increased or appeared for the first time after liming. Diatoms occurred, but were not important to total biomass until after liming when *Cyclotella* cf. *glomerata* appeared. By biomass, the most important species that increased after liming was *Dictyosphaerium* cf. *botrytella* (Chlorophyceae). Green algae as well as chrysophytes did, however, occur in all three response groups (increasing, decreasing, and no response). Apart from the cryptophytes that were all favoured and the only cyanophyte, which disappeared, there were no general trends of change within phylogenetic groups. As regards cell size there were no consistent trends in any direction, i.e., large as well as small species occurred in all three response groups. The same was also true for cell organization type (e.g., flagellates or coccoids) and potential mixotrophy. Two heterotrophic flagellate genera, *Monas* spp. (Chrysophyceae) and *Katablepharis ovalis* (Cryptophyceae), closely related to algae and for this reason counted among phytoplankton in this study, showed significant increases and appeared for the first time, respectively, after liming. In terms of edibility, calculated according to Olofsson et al. (1988), the percentage of favourable food items for zooplankton in the total biomass of phytoplankton did not change significantly after liming.

On an annual basis, bacterioplankton biomass did not change significantly after liming (Table 5). However, when only the open water periods were compared, a significant ($n = 10$, $p < 0.01$) increase in biomass was found. No measurement before liming was higher than the corresponding measurement after liming.

Protozoan (ciliate + heliozoan) biomass remained unchanged after liming as did the species diversity in this group (Table 6). Five taxa always contributed to more than 90% of the total biomass over the 2 years. Of these taxa, *Urotricha sp. 1 + Balanion sp.*, which could not be separated during routine counting, decreased significantly after liming, while the other taxa remained unchanged.

Metazoan zooplankton biomass increased by 27% after liming, while the species diversity in this group remained unchanged (Table 7). The biomass increase was primarily due to an increase in the biomass of the dominant species, *Eudiaptomus gracilis* (Copepoda), which on an annual average constituted about 70% of the total biomass of zooplankton during both years. In L. Njupfatet, *E. gracilis* forms one cohort per year. There were no differences in fecundity or initial population numbers of *E. gracilis* between the 2 years, indicating that the difference in biomass was due to a better survival of the animals during the copepodite development in July–August 1990. Three other zooplankton species (*Cyclops scutifer*, *Eubosmina longispina*, and *Polyarthra vulgaris*) also showed significant biomass increases after liming while two species (*Heterocope appendiculata* and *Holopedium gibberum*) decreased. In terms of biomass, these changes were small and had probably no major ecological consequences. None of the subdominant species showed increased fecundity after liming. Six species did not change their standing stock significantly after liming.

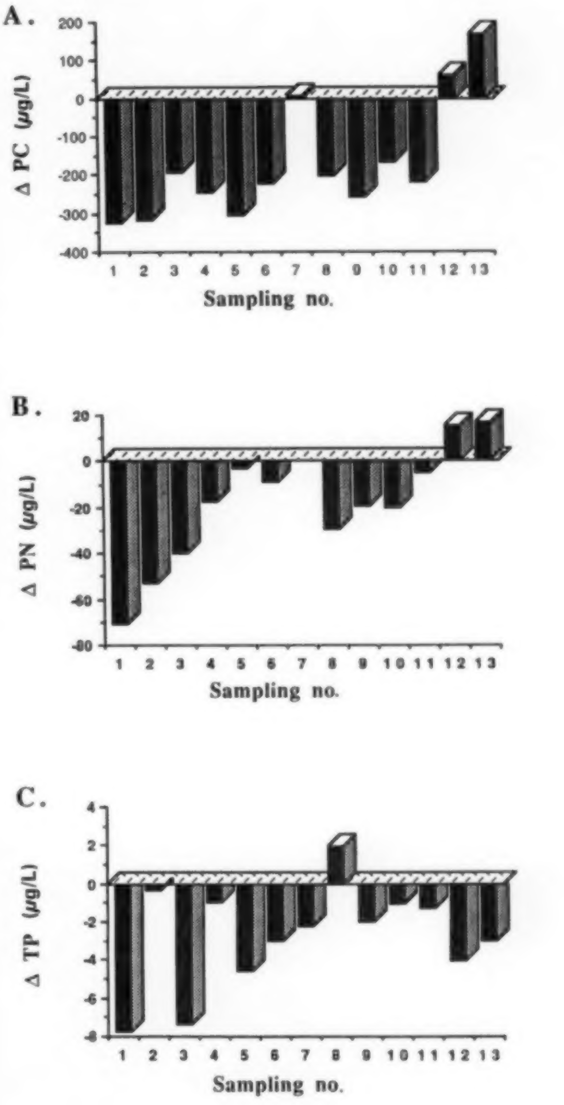


Fig. 3. The decrease in particulate carbon (A), particulate nitrogen (B), and total phosphorus (C), expressed as the difference between years (after–before liming) calculated from paired samplings in L. Njupfatet in 1989 and 1990. The lake was limed during autumn circulation. Sampling 1–3 were performed during winter with 2-month intervals, sampling 4–13 were performed biweekly during the period May–September.

Total plankton community carbon (Fig. 4) did not change significantly after liming (−5%, ns). The decrease in calculated phytoplankton carbon (−47%, $p < 0.05$) was even more pronounced than the decrease in phytoplankton wet weight (Table 3), due to the loss of the dominating, presumably carbon-rich (cf. Methods), cyanophyte. However, this loss of phytoplankton carbon was partly compensated for by a significant (+27%, $p < 0.05$) increase in metazoan zooplankton carbon, and partly by a slight and not significant increase (+12%, $p > 0.05$) in bacterioplankton carbon. Thus, in terms of total plankton community carbon the liming led to a redistribution between the different major groups from an annual average before liming of

Table 4. Phytoplankton in lake Njupfatet before and after liming.

Parameter	Before liming (1989)	After liming (1990)	Change (%)	Significance level
Total biomass (µg/L w.w.)	314±196	217±139	−31	$p < 0.05$
Shannon index H' (biomass)	2.70±0.45	2.99±0.35	11	ns
Shannon index H' (numbers)	1.57±1.11	2.20±0.65	40	ns
Taxon biomass (µg/L w.w.)				
Monas spp. (3–6 µm)	1±1	4±3	+	$p < 0.05$
Spiniferomonas sp.	0±0	3±5	+	ns *
Cyclotella cf. *glomerata*	0±0	3±7	+	ns *
Sphaerocystis schroeteri	0±0	2±6	+	ns *
Dictyosphaerium cf. *botrytella*	2±3	32±49	+	$p < 0.01$
Cryptomonas marssonii	3±5	9±8	+	$p < 0.05$
Rhodomonas lacustris	0±0	4±14	+	ns *
Katablepharis ovalis	0±0	8±10	+	$p < 0.01$
Merismopedia tenuissima	89±132	0±0	−	$p < 0.01$
Mallomonas fastigata	45±49	29±23	−	$p < 0.05$
Chrysidiastrum catenatum	7±15	0.3±0.3	−	$p < 0.05$
Monoraphidium dybowski	5±11	0.3±0.6	−	$p < 0.01$
Gymnodinium uberrimum	32±27	8±12	−	$p < 0.05$
Ochromonadaceae spp. (3–4 µm)	34±25	24±18	0	ns
Ochromonadaceae spp. (5–10 µm)	33±28	26±24	0	ns
Dinobryon crenulatum	7±14	12±18	0	ns
D. sociale var. *americanum*	2±3	3±7	0	ns
Kephyriopsis entzii	0.2±0.3	3±4	0	ns
Mallomonas sp.	1±3	5±14	0	ns
Oocystis cf. *parva*	14±16	15±14	0	ns
Ankistrodesmus nannoselene	1±1	3±3	0	ns
Gymnodinium cf. *lacustre*	24±21	12±15	0	ns

Note: Volume weighted values representing the 0–12 m water column. Mean values ± standard deviations ($N = 13$). Significance level refers to Wilcoxon's signed rank test of differences between years (limed–unlimed). *Species only found in 1990 but at too few occasions to be tested. +, positive response; −, negative response; 0, no response.

Table 5. Bacterioplankton in L. Njupfatet before and after liming.

Parameter	Before liming (1989)	After liming (1990)	Change (%)	Significance level
Total biomass (µg C/L)	31±16	35±9	12	ns

Note: Volume weighted values representing the 0–12 m water column. Mean values ± standard deviations ($N = 13$). Significance level refers to Wilcoxon's signed rank test of differences between years (limed–unlimed).

121 µg C/L distributed between the groups in the following way: bacterioplankton C, 26%; phytoplankton C, 38%; protozoan zooplankton C, 2%; metazoan zooplankton C, 34% to an annual average after liming of 114 µg C/L with a distribution between groups of 31, 21, 2, and 46%, respectively.

Discussion

In this study, we attempted to utilize a part of the National Swedish Lake Monitoring data set as a reference to chemical manipulations (standard calcite treatment) in L. Njupfatet. Evidently this set of data is limited, both with regards to the number of parameters studied and to the resolution in time, compared with that from L. Njupfatet. However, the material can be utilized to distinguish effects of liming from normal year-to-year variations in conductivity and pH, thereby indicating whether the lime treatment was successful, as well as in some ecosystem-related key water chemical parameters (e.g., TP, TN). It should also be noted that by using this reference data set, we have most likely maximized the variability as a result of sampling and analysis procedures, since the reference data set has been sampled and analysed by different persons both within and between lakes. Particularly, the high variability in total biomass of phytoplankton in one of the reference lakes

Table 6. Protozoan zooplankton in L. Njupfatet before and after liming.

Parameter	Before liming (1989)	After liming (1990)	Change (%)	Significance level
Total biomass (μg/L w.w.)	12±10	12±13	0	ns
Shannon index *H′* (biomass)	1.43±0.84	1.08±0.72	−24	ns
Shannon index *H′* (numbers)	0.95±0.60	1.09±0.69	15	ns
Taxon biomass (μg/L w.w.)				
Urotricha sp. 1 + *Balanion* spp.	4±3	1±3	–	0.05
Strobilidium cf. *velox*	2±2	1±2	0	ns
Strombidium sp.	3±3	4±4	0	ns
Oligotricha spp.	2±2	4±8	0	ns
Heliozoa spp.	1±3	1±2	0	ns

Note: Volume weighted values representing the 0–12 m water column. Mean values ± standard deviations ($N = 13$). Significance level refers to Wilcoxon's signed rank test of differences between years (limed–unlimed).

Table 7. Metazoan zooplankton in L. Njupfatet before and after liming.

Parameter	Before liming (1989)	After liming (1990)	Change (%)	Significance level
Total biomass (μg/L d.w.)	86±13	109±34	27	$p < 0.05$
Shannon index *H′* (biomass)	1.36±0.43	1.34±0.43	−1	ns
Taxon biomass (μg/L d.w.)				
Eudiaptomus gracilis	61±9	78±21	+	$p < 0.01$
Cyclops scutifer	5±2	9±3	+	$p < 0.01$
Eubosmina longispina	4±4	9±8	+	$p < 0.05$
Polyarthra vulgaris	0.2±0.1	0.8±0.9	+	$p < 0.01$
Heterocope appendiculata	7±7	1±2	–	$p < 0.01$
Holopedium gibberum	0.1±0.2	0±0	–	$p < 0.01$
Cyclops sp.	2±3	3±2	0	ns
Diaphanosoma brachyurum	4±5	5±9	0	ns
Kellicottia longispina	1.4±1.8	1.2±1.9	0	ns
Conochilus unicornis	0.4±0.8	0.8±1.1	0	ns
Keratella hiemalis	0.02±0.04	0.03±0.03	0	ns
Other rotatoria	0.01±0.02	0.02±0.06	0	ns

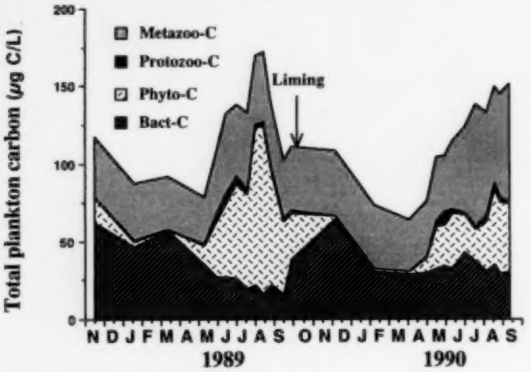
Note: Volume weighted values representing the 0–12 m water column. Mean values ± standard deviations ($N = 13$). Significance level refers to Wilcoxon's signed rank test of differences between years (limed–unlimed).

(+347%) is most likely due to the change of analysis' personnel between the 2 years. In most cases, normal year-to-year variations in total phytoplankton biomass keeps within a factor of 2 of the minimum value (cf. Ramberg 1979; Olsson et al. 1992). We thus consider our evidence that liming, apart from increasing pH and conductivity, led to a decrease in TP, an increase in NO_3-N, and an increase or rather, when compared with the reference lakes, lack of decrease in TN, as relatively strong. The decrease in total phytoplankton biomass in L. Njupfatet after liming was significant at the $p < 0.1$ level and we believe that this result should be taken into consideration as it includes the extinction of the dominant species, which was not the case in any of the reference lakes.

According to Swedish guidelines (Bernes 1991), the ordered "standard liming" of L. Njupfatet was successful in so far that pH and alkalinity increased to above 6 and above 0.1 mequiv./L, respectively. As pH increased to 7.3, the liming may even be considered slightly "oversuccessful" (defined as an increase in pH to more than 0.3 units over calculated preindustrial values, Bernes 1991) since pH in L. Njupfatet on the basis of diatom inferred pH reconstruction did not exceed 6 before air-borne acidification started (N.J. Anderson, P. Blomqvist, and I. Renberg, in preparation). Oversuccessful limings are, however, quite common in the Swedish liming program and have been found in some 31% of all cases (1800 lakes, Bernes 1991).

The liming led to considerable changes in the water chemistry and pelagic community structure in the lake. TIN increased due solely to increased concentrations of NO_3-N. The increase in NO_3-N concentrations could be due either to increased decomposition of organic nitrogen,

Fig. 4. Total plankton community carbon and the relative contribution of bacterioplankton, phytoplankton, protozoan zooplankton, and metazoan zooplankton in L. Njupfatet before (1989) and after liming (1990).



coupled with nitrification, or decreased biological uptake of NO_3-N after liming. Nitrification is known to cease when pH drops below about 6 (Nygaard 1938; Fenchel and Blackburn 1979; Rudd et al. 1988) and, if the relationship between pH and nitrification is direct, liming alone should be enough to reverse this process. The slight but significant increase in DOC indicates improved degradation properties in the lake basin as a whole and supports the view that the decomposition or organic nitrogen increased. Increased decomposition of organic material after liming could be expected as acidification is believed to lead to reduced decomposition rates and accumulation of particulate material (Grahn et al. 1974; Andersson 1985). It is, however, interesting to note that such a reasoning, including mineralization of particulate matter and subsequent release of dissolved compounds, is not applicable for phosphorus, which is discussed further below.

TP decreased significantly after liming, contrary to most other studies that indicate unaltered or increased phosphorus concentration (cf. Introduction). However, using regional data sets from nonacidified lakes, acidified lakes, and limed lakes, Olofsson et al. (1988) concluded that limed lakes usually had lower concentrations of TP than the nonacidified lakes of corresponding pH, although such analyses provide no evidence of cause and effect (cf. Olsson and Pettersson 1993). Decreased concentrations of phosphorus within a lake after lime treatment were also reported by Molot et al. (1990*a*), these authors attributed the decrease to coprecipitation of phosphate ions by calcium ions, increased sedimentation of phytoplankton or "most likely to contamination of early samples." In view of the fact that three strata were each sampled 19 times before neutralization ($n = 57$) the latter conclusion seems unfathomable.

The reduction in TP in L. Njupfatet was principally due to a decrease in PP and accompanied by a corresponding (−30%) decrease in PC and PN, suggesting that the lower concentrations after liming were due to particle sedimentation. Since only about 20% of the particulate organic carbon was tied to living organic material, which did not change significantly after liming, the reduction in PC, PN, and PP was mainly due to sedimentation of dead particulate organic matter. In view of the long retention time of the water in L. Njupfatet, reflected by the extreme clarity of the water, it seems likely that the particles were not to any greater extent of allochthonous origin (e.g., humic matter) but instead produced in the lake (e.g., planktonic organisms and their remnants). As the removal of particles did not alter the C:N:P ratio of the particulate matter in the lake, this indicates either a rather constant proportion between these elements in all particles or, more likely, a sedimentation of equal proportions of all kinds of particles.

It is interesting to note that the average (comparing the means in Table 3) DOC:DOP and DON:DOP ratios doubled, while the DOC:DON ratio remained constant after liming (even though the changes were not statistically testable because DOP was often below the detection limit, which made the quotient at some individual sampling occasions unreasonably high). This decrease of the phosphorus content in the dissolved organic matter (DOM) is another indication of increased decomposition in the lake after liming. It has been shown experimentally that in the acidified L. Njupfatet during 1985 and 1986, as well as in other acidified headwater lakes, there was a close balance between nitrogen and phosphorus limitation of phytoplankton production (Blomqvist et al. 1989). Further evidence was found that phosphorus was not the sole limiting factor for phytoplankton production in enclosure experiments in L. Njupfatet during 1989 (the year before liming) as phosphate additions alone did not lead to any response in the ATP level of phytoplankton samples (Pettersson and Blomqvist 1992). In an identical experiment performed after liming, phytoplankton responded to phosphate treatment by a significantly increased ATP level, indicating phosphorus limitation. Furthermore, phosphatase activities in lake water were low before liming, while after liming the acid and alkaline phosphatase activities increased by 1200 and 2400%, respectively (Pettersson and Blomqvist 1992).

Another noticeble feature is that if liming induced phosphorus limitation of phytoplankton growth, from co-limitation by phosphorus and nitrogen, the decline in TP of 2.7 µg P/L after liming would correspond to an increase in the pool of inorganic nitrogen of 19 µg N/L (using the Redfield N:P ratio in algal cells of 7:1). In L. Njupfatet, the pool of inorganic nitrogen increased to 15 µg N/L after liming.

After liming there was an almost uniform decrease in the particulate fractions of carbon, nitrogen, and phosphorus (PC, PN, and PP). The decrease in PP was accompanied by a similar decrease in TP, whereas the decreases in PN and PC did not result in similar decreases in TN or TOC. The loss of PC and PN, but not PP, was compensated for by increases in the dissolved fractions and therefore TN and TOC remained stable. As a result, there were significant increases in the TOC:TP and TN:TP ratios in the lake water. Since particles were lost it seems likely that the loss was due to sedimentation. The increase in TOC and TN can be explained by mineralization and subsequent release of dissolved carbon and nitrogen to the water column. However, this was not the case for phosphorus. The decrease of PP was not followed by a proportionate increase in the dissolved pool, which indicates that phosphorus was either chemically precipitated or biologically taken up in the bottom area. Co-precipitation of organic particles and subsequent mineralization and release of inorganic constituents may explain why the added lime could lead to precipitation of phosphorus which, from a stoichiometic point of view, is unlikely to occur in the water (Stumm and Morgan 1970). In sediment cores from L. Njupfatet, taken for analysis of diatom frustules (P. Blomqvist, unpublished), a lime layer on top of the sediments was clearly visible. If remineralized, inorganic phosphorus from organic particles precipitated by the lime and from deeper sediment layers would have to penetrate a lime layer before reaching the open water. In such an environment, precipitation of inorganic phosphorus is likely to occur. From a general point of view, the anoxic conditions prevailing in deeper strata during summer 1990 should decrease the capability of the sediments to act as phosphorus traps compared with the situation before liming (1989) when oxygen conditions were better. In spite of this, the phosphorus concentrations in the lake water decreased which further stresses that the difference between years was caused by liming.

It may seem paradoxical that, if phosphorus was limiting to the primary producers, the total plankton community biomass did not decrease significantly after liming when the pool of phosphorus decreased by 30%. One explanation to this lack of decline is that the amount of particulate matter in living organisms was low and that changes in the total particulate matter will therefore not be reflected in biota, which could obtain nutrients from nonliving particles and DOM. It may also be explained by a restructure within the biotic community, namely the decrease in phytoplankton carbon and the increase in zooplankton carbon (mainly as an increase in *Eudiaptomus gracilis*). Assuming that the Redfield ratio of C:N:P is valid for phytoplankton and that the average content of these elements of 129:26:1 for *E. gracilis* (Hessen and Lyche 1991) is valid for zooplankton, the observed shift would mean that the pool of phosphorus necessary to sustain the same combined biomass of phyto- and zoo-plankton was 0.5 μg P/L lower after than before liming.

At the species level, the most dramatic biotic change in L. Njupfatet after liming was the total disappearance of the dominant phytoplankton species in the lake during the years prior to liming, the cyanophyte *Merismopedia tenuissima*. From what has been found in other limed lakes (cf. Introduction), this change was expected, suggesting that this species is a good indicator of moderatly acidified conditions (Brettum 1989; Nicholls et al. 1992). Other important changes were the decrease in phytoplankton biomass (in agreement with what was suggested by Olofsson et al. 1988) and the increased relative importance of bacterioplankton and metazoan zooplankton in the pelagic community. From an ecosystem point of view this means that liming altered the plankton community structure from the inefficient phytoplankton- (*Merismopedia*) dominated plankton community to the more efficient, zooplankton and decomposer dominated, plankton community typical of undisturbed oligotrophic lakes (Olofsson et al. 1988; Havens 1992). However, as the proportion of unfavourable food items for zooplankton in the phytoplankton community remained unaltered (instead of decreased as hypothesized by Olofsson et al. 1988) such a conclusion may be a bit hasty, especially against background of the lack of change in the zooplankton community structure and since the increase in zooplankton biomass was not due to increased reproduction but instead to better survival during late summer. Concerning continued dominance by calanoids, our results support the conclusion by Stensson et al. (1993) that liming has little effect on crustacean community structure at least in the short term perspective.

As regards the effects of liming on the microbial food web (bacteria, protozoan zooplankton) our data indicate little, if any, response at the biomass levels. However, there were several indications of increased productivity in this part of the food web indicating that the lack of biomass response may have been caused by increased elimination. First of all, the water chemistry data indicate increased bacterial activity as, for example, DOC and TIN increased. Secondly, if only the bacterial samples from the open water period are taken into consideration, bacterioplankton biomass increased very significantly. Small (<10 μm) predators on bacteria such as *Katablepharis ovalis* (Cryptophyceae) and *Monas* spp. (Chrysophyceae), both heterotrophic flagellates, did increase significantly after liming. As the standing stock of *Eudiaptomus gracilis* increased significantly, the lack of response by protozoan zooplankton may also be explained by predation. Experimental data (U. Stensdotter unpublished) clearly indicate that these organisms were under predator control by *E. gracilis*.

In contrast with what has been found in Swedish west-coast lakes (e.g., Appelberg et al. 1990; Hörnström et al. 1993), and which has been generalized to be valid for the entire Swedish liming program, our data do not indicate that liming has "favourable effects on biota almost without exception" (Bernes 1991), i.e., leads to a restoration of acidified lakes rather than to the formation of a new type of ecosystem. In the limed L. Njupfatet, phosphorus concentrations decreased in comparison to before liming as did phytoplankton biomass. Phytoplankton community structure did not improve as regards suitable food items for higher trophic levels. Zooplankton community structure did not change but remained "the simple acid-state crustacean zooplankton community, with dominance of calanoids" which according to Stensson et al. (1993) "may continue some years after liming." If liming leads to a reduction of the phosphorus levels in the water during the first 1–2 years after treatment and with the subsequent effects on biota described above, this implies that during 20–40% of the expected duration (5 years) of a standard lime treatment, the "favourable effects on biota" will be limited. In such lakes liming may be complemented with controlled additions of nutrients in order to widen the food base and to avoid the loss of predator levels (cf. Olofsson et al. 1988; Blomqvist et al. 1993).

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Stock-recruitment models for Pacific sardine (*Sardinops sagax*)[1]

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Abstract: We used generalized additive models to study the recruitment of Pacific sardine (*Sardinops sagax*) along western North America and to compare the effects of fishing and environmental factors on the stock. We found significant relationships between the logarithm of sardine reproductive success (recruits per unit of spawning biomass) and average sea surface temperature (SST), as well as between sardine recruitment, spawning biomass, and average SST. Simulation and time series analyses were used to evaluate bias, variance, and statistical power for one of our models. Correlation with log reproductive success was highest when average SST data included temperatures after the period of larval development, indicating that recruitment estimates were affected by environmentally driven changes in availability of older sardine to the fishery. Predicted equilibrium biomass and MSY for sardine are lower under environmental conditions that prevail when temperatures are colder. Long-term fluctuations in the environment may cause long-term fluctuations in sardine productivity, and little or no sardine harvest may be sustainable during periods of adverse environmental conditions and cold temperatures.

Résumé : Nous avons eu recours à des modèles additifs généralisés pour étudier le recrutement de la sardine du Pacifique (*Sardinops sagax*) le long de la côte ouest de l'Amérique du Nord, et pour comparer les effets de la pêche et des facteurs environnementaux sur le stock. Nous avons trouvé des relations significatives entre le logarithme du succès de reproduction de la sardine (nombre de recrues par unité de biomasse reproductrice) et la température moyenne à la surface de la mer (SST), ainsi qu'entre le recrutement des sardines, la biomasse reproductrice et la SST moyenne. La simulation et les analyses des séries chronologiques ont servi à évaluer le biais, la variance et la puissance statistique de l'un de nos modèles. La corrélation avec le logarithme du succès de reproduction était la plus haute quand les données sur la SST moyenne incluaient les températures après la période de développement larvaire, ce qui indique que les estimations du recrutement étaient affectées par des changements d'origine environnementale dans la capturabilité des sardines plus âgées. On prédit une biomasse à l'équilibre et une production maximale équilibrée plus faibles dans les conditions environnementales qui prévalent en cas de températures plus basses. Les fluctuations environnementales à long terme peuvent se répercuter sur la productivité des sardines, et il peut être justifié dans une perspective durable de ne prélever pratiquement aucune sardine pendant les périodes où les conditions environnementales sont néfastes et où les températures sont basses.

[Traduit par la Rédaction]

Introduction

The Pacific sardine (*Sardinops sagax*) fishery off the west coast of North America was once the largest in the western hemisphere with peak landings of 720 000 t during 1936 (Schaefer et al. 1951; Radovich 1982; Wolf and Smith 1992). As landings peaked, sardine biomass (3.5 million t in 1932) began to decline and fell to less than 10 000 t by 1965 (Murphy 1966; MacCall 1979). The stock did not increase noticeably until the early 1980s. Current estimates of sardine biomass are on the order of 100 000–200 000 t (Barnes et al. 1992; Deriso 1993).

Debate about effects of overfishing, environmental factors, contaminants, and interspecific interactions on the sardine stock has continued for decades (Clark and Marr 1955; Murphy 1960; Radovich 1962; Marr 1963; MacGregor 1964; Murphy 1966; Garrod 1982; Radovich

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1982; Prager and MacCall 1993). Environmental factors, particularly water temperatures, have long been thought to affect sardine population dynamics (Marr 1960). Barnes et al. (1992) showed that high sardine biomass occurred when water temperatures were warm and fishing mortality rates low, while declines occurred when water temperatures were cold and fishing mortality rates high. Butler et al. (1993) concluded that water temperatures can affect sardine recruitment by affecting the duration of egg and larval stages. According to Murphy (1960), larval mortality rates for sardine may be greater when temperatures are low because of increased predation by zooplankton. Upwelling, associated with cold water, may limit sardine recruitment because of advection of larvae into offshore areas where survival is poor (Murphy 1960). Effects of poor environmental conditions on recruitment of sardine may be exacerbated by low growth rates in new recruits associated with poor reproductive success (Iles 1973). Sardine spawning may tend to be unsuccessful in cold water because spawning is delayed or the spawning period is shortened (Marr 1960).

The purpose of this paper is to demonstrate effects of spawning biomass and sea surface temperature (SST) on sardine recruitment. We describe recruitment models for sardine, statistical methods, data, and use results to estimate surplus production of sardine over a range of temperature and biomass levels. We assess the relative importance of environmental and density dependent effects on sardine recruitment and formulate a new hypothesis about the link between temperature and recruitment.

Models

We investigated environmental and density dependent effects on sardine reproduction using generalized additive models (Hastie and Tibshirani 1990). The model for recruitment was

$$[1]\quad \ln(R) = \alpha + f(T) + g(S) + \epsilon$$

where R is recruitment in number of sardine (age 2), α is an intercept parameter, $f(T)$ is a function of the environmental variable T, $g(S)$ is a function of spawning biomass (S), and ϵ is a normally distributed random variable. The log transformation stabilizes the variance of residuals and can be justified on biological grounds (Hilborn and Walters 1992).

The model for reproductive success was derived from a linearized Ricker (1975) recruitment model modified to include an environmental variable (Prager and MacCall 1993) and a generalized relationship to spawning biomass:

$$[2]\quad \ln\left(\frac{R}{S}\right) = \alpha + h(T) + j(S) + \epsilon$$

where $h(T)$ and $j(S)$ are functions of the environmental variable and spawning biomass. Models for reproductive success are widely used but spurious correlations may arise because the independent variable S also occurs in the dependent variable as the denominator of a ratio (Atchley et al. 1976).

Components in general additive models [$f(T)$, $g(S)$, $h(T)$, and $j(S)$] can take linear or nonlinear forms. Nonlinear

Table 1. Spawning biomass, recruitment, and SST data for sardine by fishing season.

Fishing season	Spawning biomass (10^3 t)	No. of recruits (10^6 fish)		Mean three season SST (°C)	Mean five season SST (°C)
1935	3526	4098		16.56	16.78
1936	3417	2821		16.84	17.02
1937	3628	5383		17.11	16.96
1938	2847	6940		17.12	17.14
1939	1689	6763		16.98	17.30
1940	1207	11808		17.15	17.20
1941	1202	14442		17.31	17.19
1942	1609	6152		17.50	17.28
1943	1761	3268		17.21	16.94
1944	2459	3720		16.94	16.79
1945	2066	2385		16.72	16.81
1946	1679	1625		16.64	16.67
1947	1261	1667		16.72	16.54
1948	720	3875		16.76	16.57
1949	566	4261		16.51	16.53
1950	405	3690		16.31	16.43
1951	740	290		16.41	16.37
1952	793	397		16.55	16.46
1953	780	972		16.43	16.49
1954	277	1197		16.39	16.42
1955	136	382		16.41	16.52
1956	202	264		16.50	16.86
1957	239	588		16.65	17.15
1958	170	1586		17.04	17.35
1959	108	905		17.62	17.39
1960	90	288		17.80	17.23
1961	177	111		17.39	16.99
1962	122	74		16.77	16.74
1963	88	56		16.43	16.51
1985			(131)	17.80	17.76
1986	5	88	(85)	17.87	17.68
1987	18	57	(317)	17.71	17.44
1988	24	212	(240)	17.54	17.39
1989	33	161	(355)	17.23	17.37
1990	56	238		17.18	17.39

Note: Historical spawning biomass for 1935–1963 is sardine age 2 and older. Recent spawning biomass for 1985–1990 is sardine age 1 and older. Reliable spawning biomass and recruitment estimates for sardine during 1964–1984 are not available (Barnes et al. 1992). Recruitment is the number of age 2 fish (millions) on July 1 of the fishing season indicated. Recent recruitment estimates derived from original data as described in the text (original data in parenthesis). For example, 5 000 t of sardine spawned during 1983 and produced 131 million age 1 sardine that recruited on 1 July 1985 or equivalently as 131 × 0.67 = 88 million 2-year-olds on 1 July 1986.

Table 2. Correlations between log reproductive success for sardine and mean SST at Scripps Pier as a function of the first and last fishing seasons used to compute mean SST.

First season in mean	Last season in mean												
	$r-6$	$r-5$	$r-4$	$r-3$	$r-2$	$r-1$	r	$r+1$	$r+2$	$r+3$	$r+4$	$r+5$	$r+6$
$r-6$	−0.19	−0.15	−0.07	0.02	0.12	0.23	0.31	**0.34**	**0.34**	0.32	0.29	0.18	0.14
$r-5$		−0.08	0.01	0.11	0.20	0.32	**0.39**	**0.42**	**0.41**	**0.39**	**0.36**	0.26	0.22
$r-4$			0.11	0.19	0.28	**0.40**	**0.47**	**0.50**	**0.47**	**0.44**	**0.41**	0.32	0.28
$r-3$				0.23	0.32	**0.45**	**0.52**	**0.54**	**0.50**	**0.46**	**0.43**	**0.33**	0.29
$r-2$					**0.34**	**0.48**	**0.55**	**0.55**	**0.50**	**0.46**	**0.42**	0.32	0.27
$r-1$						**0.50**	**0.54**	**0.53**	**0.46**	**0.41**	**0.37**	0.27	0.20
r							**0.43**	**0.41**	**0.34**	0.30	0.26	0.15	0.08
$r+1$								0.28	0.22	0.19	0.15	0.03	−0.04
$r+2$									0.10	0.10	0.08	−0.04	−0.11
$r+3$										0.10	0.07	−0.04	−0.12
$r+4$											0.07	−0.06	−0.14
$r+5$												−0.08	−0.17
$r+6$													−0.18

Note: For each cohort in the data, the fishing season when recruitment at age 2 occurred is season r, $r + n$ is n seasons after recruitment, and $r - n$ is n seasons prior to recruitment. For example, the value in row $r - 3$ and column $r + 1$ (0.54) is the correlation between log reproductive success and mean SST computed $T = (t_{r-3} + t_{r-2} + t_{r-1} + t_r + t_{r+1})/5$. Thirty-four observations were used to compute correlations in columns $r - 6$ to $r + 2$, 33 for column $r + 3$, 32 for column $r + 4$, 31 for column $r + 5$, and 30 for column $r + 6$. Correlations nominally significant at the $p = 0.05$ level are boldface.

components are fit using "scatterplot smoothers" (Härdle 1990) and it is possible to mix linear and nonlinear components in the same model. We used Cleveland et al.'s (1988) locally weighted scatterplot smoothing estimator (called "loess" in Cleveland et al. 1988 and Hastie and Tibshirani 1990) and the S-plus software package (Statistical Sciences Inc., 1700 Westlake Ave. N., Suite 500, Seattle, WA 98109) to fit nonlinear components in models for sardine. Loess components were fit by specifying a neighborhood size (0.75 or 0.5 of the total number of observations worked well for our data) and then fitting a weighted linear or quadratic regression to data in the neighborhood of each observation. Larger neighborhoods and linear fits give smoother regression lines but potentially more bias while smaller neighborhoods and local quadratic fits give less bias but rougher regression lines. Approximate 95% confidence intervals for fitted values from our models (twice the pointwise standard errors for each observation) were calculated as described in Hastie and Tibshirani (1990).

We used a manual model selection process based on approximate F-tests (Hastie and Tibshirani 1990) to select the best predictive model for our sardine data. We rejected a smaller model (with fewer parameters) in favor of a larger model if an F-test indicated that the reduction in residual sums of squares using the larger model was significant (Draper and Smith 1981). Relationships between model size, smoothness, and degrees of freedom are important in the context of F-tests and model selection with generalized additive models. Nonlinear model components based on large neighborhoods and local linear fits give smoother lines and use fewer degrees of freedom than model components based on smaller neighborhoods and local quadratic fits. In addition, linear model components are smaller, smoother, and use fewer degrees of freedom than nonlinear fits. Thus, we were able to use F-tests to decide which variables were useful for predicting sardine recruitment and the most appropriate type of model component (linear or nonlinear) for each variable. Residual plots were used to check model fit (Weisberg 1980).

Data

We combined two types of spawning biomass and recruitment data for sardine (Table 1). All data were organized by fishing seasons (July to June) that correspond to the annual biological cycle for sardine because recruitment occurs on approximately July 1. For example, the 1935 fishing season began on 1 July 1935 and ended on 30 June 1936. "Historical" data (1935–1963) were obtained by virtual population analysis (Murphy 1966; MacCall 1979) and "recent" data (1986–1990) from CANSAR (catch-age-analysis for sardine), which was developed especially for sardine by Deriso (1993). Recent data were important because they provide information about sardine recruitment at low biomass levels and warm temperatures that is not available in the historical data. We eliminated the last 2 years of data from each source because the most recent biomass and recruitment estimates from catch-at-age analyses are unreliable (Rivard 1989).

The CANSAR model was similar to CAGEAN (Deriso et al. 1985) and included three types of fishery independent auxiliary information about trends in sardine abundance. It was not possible to use CANSAR to re-estimate spawning biomass and recruitment of sardine during the historical period because little auxiliary information about sardine abundance was available.

Recent and historical recruitment data for sardine were not immediately comparable because recruitment occurred at age 2 in the historical data and at age 1 in the recent data. We multiplied recent recruitment data for age 1 sardine by exp($-M$), where $M = 0.4$/year is the natural mortality rate for sardine assumed in all studies (MacCall 1979; Deriso 1993), to convert recent recruitment data to equivalent number of recruits at age 2. Models were fit to historical and recent data and then to historical data only to assess sensitivity of results to differences in the two types of sardine data. Most of the spawner biomass data we used were derived from virtual population analyses that can give spurious correlations between recruitment and environmental variables because of errors in the assumed natural mortality rate and trends in fishing mortality (Lapointe and Peterman 1991).

We used daily SST (°C) at Scripps Pier (near San Diego, Calif.) as the environmental variable (T) in our analyses because it was readily available and because previous studies suggested a relationship between SST at Scripps Pier and changes in sardine abundance (Radovich 1982; Barnes et al. 1992). Temperature might not be the most important environmental factor affecting sardine recruitment but SST at Scripps Pier is useful as a "proxy" or "surrogate" variable (Marr 1960) and is also correlated with other environmental variables, such as upwelling, salinity, and sea level (Prager and MacCall 1986; Prager and MacCall 1993) that might be more important.

We conducted exploratory correlation analyses to help determine how SST data should be aggregated for use in our models. Correlations between log reproductive success for sardine (ln(R/S) in [2]) and average SST were calculated for a range of first and last fishing seasons used in computing the average. Highest correlations (>0.5, Table 2) were obtained using averages that included SST data for fishing seasons when recruits were 1–4 years old. These results are in contrast with current theory, which hypothesizes that environmental factors during the short period of early life-history stages account for most of the variation in recruitment (Hjort 1914; Murphy 1960; May 1974; Lasker 1981).

We tried to avoid spurious correlations between recruitment and environmental variables (Shepherd et al. 1984) by focusing on a single environmental variable considered important a priori, and by using averages over periods that made sense biologically. Guided by exploratory analysis, theory, and practical issues, we used two sets of mean SST data as the environmental variable in our models. The first set of mean SST data was averaged over the three fishing seasons prior to recruitment, i.e., $T_r = (t_{r-3} + t_{r-2} + t_{r-1})/3$ where T_r is the mean SST value used to predict recruitment of sardine at age two in fishing season r, and t_i is mean Scripps Pier SST during fishing season i. The second set was averaged over the period beginning three fishing seasons prior to recruitment and ending in the fishing season 1 year after recruitment when the cohort was age three, i.e., $T_r = (t_{r-3} + t_{r-2} + t_{r-1} + t_r + t_{r+1})/5$. The first set of SST data is called "mean three season" SST data and was used in "management" models. The second is called "mean five season" SST data and used in "research" models. Management models might be used by fishery managers who wish to make short-term predictions about future recruitment on the basis of currently available data. Research models utilized all useful information to model recruitment of sardine for scientific purposes. Temperature data for periods after recruitment to the fishery were not included in management models because managers have no access to future temperature data when making decisions about the current or next fishing season. Temperatures during the three fishing seasons prior to recruitment were included in both sets of SST data because the three season period covers the life of a cohort from gametogenesis and spawning by parents, hatching, early life-history stages, and growth to recruitment at age 2. Research models included temperature data for fishing seasons after recruitment to the fishery because exploratory analysis indicated they were important (see Discussion).

SST data, while measured accurately, were subject to a type of "process" error (Hilborn and Walters 1992) because mean SST at Scripps pier is a proxy measurement of the environmental factors that actually affect sardine recruitment. Our models might fail if the relationship between SST and environmental factors affecting sardine recruitment change over time.

Error in measurement of spawning biomass (the "errors in variables" problem, Ludwig and Walters 1981), time series bias (Walters 1985), and statistical power (Peterman 1990) are important issues in recruitment studies. Errors in variables and time series bias obscure relationships between recruitment and spawning biomass and cause biased parameter estimates. Serial correlation in spawning biomass and environmental data (related to time series bias) may reduce the precision of correlation coefficients (Drinkwater and Meyers 1987) and other parameter estimates. Statistical power is important because the probability of detecting a relationship between recruitment and explanatory variables may be low even if a relationship actually exists. Problems because of measurement error are reduced when the range of spawning biomass is large (Walters and Ludwig 1981). Spawning biomass for sardine in our analysis ranged from 5000 to 3.5 million t (Table 1) so bias and other problems were probably reduced. We used simulation analyses (Walters 1985; Hilborn and Walters 1992) and time series analysis to evaluate bias, precision, and power (see Appendix).

Results

Selected management and research models [1] fit to log recruitment data for sardine were nonlinear in spawning biomass and linear in SST (Table 3 and Fig. 1). In terms of F-tests, the best models included a quadratic loess component for sardine spawning biomass with neighborhood size 0.5. We preferred, however, a linear loess on spawning biomass with neighborhood size 0.5 because it gave smoother fitted values at sardine spawning biomass levels around 400 000 t. Lack of smoothness in fitted lines was due to scarcity of recruitment data for spawning biomass levels near 400 000 t. Models with linear loess and quadratic loess components for spawning biomass gave similar results at spawning biomass levels below and above 400 000 t, and models using quadratic components were only a little better in statistical terms (Table 3).

Table 3. *F*-tests for management and research models [1] fit to log recruitment data for sardine.

Model	Residual df	Residual sum of squares	F	p value
Management models				
$\ln(R) = \alpha + \epsilon$				
$(\mathrm{Var}(\epsilon) = 2.69)$	33	88.83		
$\ln(R) = \alpha + \beta S + \epsilon$				
$(R^2 = 45\%, \mathrm{Var}(\epsilon) = 1.53)$	32	49.10	59.76	$<10^{-7}$
$\ln(R) = \alpha + g(S) + \epsilon^*$				
$(R^2 = 71\%, \mathrm{Var}(\epsilon) = 0.90)$	29.1	26.16	12.06	$<10^{-4}$
Preferred model*				
$\ln(R) = \alpha + g(S) + \beta T + \epsilon$				
$(R^2 = 76\%, \mathrm{Var}(\epsilon) = 0.76, \beta = 1.04)$	28.1	21.34	7.24	0.01
Best model†				
$\ln(R) = \alpha + g(S) + \beta T + \epsilon$				
$(R^2 = 81\%\ \mathrm{Var}(\epsilon) = 0.66, \beta = 1.22)$	25.7	17.07	2.62	0.08
Research models				
$\ln(R) = \alpha + \epsilon$				
$(\mathrm{Var}(\epsilon) = 2.69)$	33	88.83		
$\ln(R) = \alpha + \beta S + \epsilon$				
$(R^2 = 45\%, \mathrm{Var}(\epsilon) = 1.53)$	32	49.10	74.73	$<10^{-7}$
$\ln(R) = \alpha + g(S) + \epsilon^*$				
$(R^2 = 71\%, \mathrm{Var}(\epsilon) = 0.90)$	29.1	26.16	15.08	$<10^{-5}$
Preferred model*				
$\ln(R) = \alpha + g(S) + \beta T + \epsilon$				
$(R^2 = 80\%, \mathrm{Var}(\epsilon) = 0.63, \beta = 1.71)$	28.1	17.70	15.91	0.0005
Best model†				
$\ln(R) = \alpha + g(S) + \beta T + \epsilon$				
$(R^2 = 85\%, \mathrm{Var}(\epsilon) = 0.53, \beta = 2.07)$	25.7	13.66	3.11	0.05

Note: *F*-tests and *p* values determine if a model is significantly better than the smaller model listed above it. There were 34 observations.

*$g(S)$ a linear loess fit with neighborhood size 0.5.

†$g(S)$ a quadratic loess fit with neighborhood size 0.5.

Results indicate that spawning biomass is more important than SST as a predictor of log sardine recruitment because SST was not significant unless spawning biomass was already included. In addition, *p* values for spawning biomass were much smaller than *p* values for SST (Table 3). Predicted values from the models for log sardine recruitment are available from the first author.

Recent data were important for measuring temperature effects in management models for log recruitment. The best research model fit to historical recruitment data was the same form as the model fit to the entire data set (i.e., nonlinear in spawning biomass and linear in SST). The best management model fit to historical sardine recruitment data was also nonlinear in spawning biomass, but SST was not significant at the $p = 0.05$ level (Table 3).

The selected research model for log reproductive success [2] was nonlinear in SST but not significant for spawning biomass (Fig. 2 and Table 4). The shape of the curve indicates that sardine reproductive success increases when mean five season SST exceeds 16.8°C (Fig. 2). Similar results were obtained when the research model was fit to historical data only.

The selected management model for log reproductive success was linear in SST but, like the research model, not significant for spawning biomass (Table 5). Similar results were obtained when the management model was fit to historical data only. We report parameter estimates for management models with and without spawning biomass (Table 5) because the linear management models are tractable mathematically, and because estimated effects of spawning biomass might be important. Meyers et al. (1993) describe management problems that can occur when environmentally driven models are used to predict recruitment with no information about spawning biomass levels.

Management models for sardine recruitment were similar in shape, while research models were more variable (Fig. 3). For ease of comparison, results from research and management models for log recruitment [1] and log reproductive success [2] were converted to units of arithmetic recruitment at the first and third quartiles of mean Scripps Pier SST (16.5 and 17.2°C). Results were plotted for models that included both spawning biomass and SST to standardize results. For simplicity, anti-logged values were median estimates not corrected for bias due to log transformation

Table 4. *F*-tests for research models [2] fit to log reproductive success data for sardine.

Model	Residual df	Residual sum of squares	F	p value
$\ln (R/S) = \alpha + \epsilon$ $(\mathrm{Var}(\epsilon) = 1.06)$	33	35.00		
$\ln (R/S) = \alpha + \beta T + \epsilon$ $(R^2 = 29\%, \mathrm{Var}(\epsilon) = 0.77)$	32	24.79	16.12	0.0003
$\ln (R/S) = \alpha + h(T) + \epsilon$ $(R^2 = 44\%, \mathrm{Var}(\epsilon) = 0.63)$	30.9	19.55	7.26	0.009

Note: *F*-tests and *p* values as in Table 3. The nonlinear component *h(T)* for SST in the best model was a linear loss with neighborhood size 0.75. There were 34 observations.

Fig. 1. Predicted values (with 95% confidence intervals) and residuals from the preferred research model [1] for log recruitment fit to sardine spawning biomass (top panel) and mean five season SST (bottom panel) data. Predicted values and residuals are adjusted to an average value of zero.

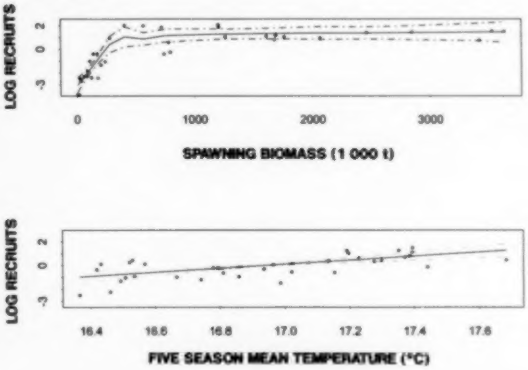
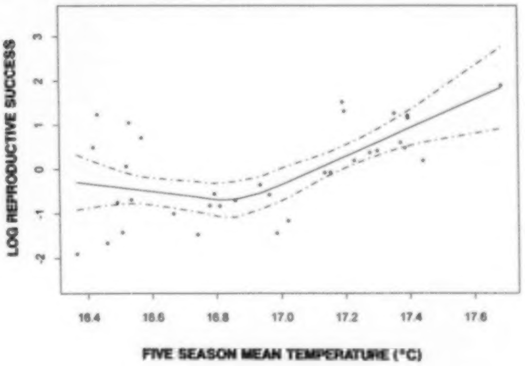


Fig. 2. Predicted values (with 95% confidence intervals) and residuals from the best research model [2] for sardine log reproductive success. Predicted values and residuals are adjusted to an average value of zero.



(Beauchamp and Olson 1973). All models indicate that sardine recruitment depends on spawning biomass (Fig. 3) and environmental factors related to temperature (Fig. 4).

Sardine spawning biomass was significant in the research and management models for log recruitment [1] only, while SST was significant in the research and management models for both log recruitment [1] and reproductive success [2]. Some care must be exercised in interpreting these results. As is evident in Fig. 4, both models indicate that sardine recruitment depends on spawning biomass and environmental factors associated with SST. Model [2] tests whether log reproductive success (not recruitment per se) is related to spawning biomass; lack of significance indicates that the null hypothesis of constant log reproductive success for sardine could not be ruled out. Recruitment in numbers of fish calculated using predicted values from model [2], is the product of reproductive success (estimated as a constant by model [2]) and spawning biomass. Because recruitment is the product of spawning biomass and reproductive success, effects of spawning biomass on recruitment are substantiated by both models.

Models for log sardine recruitment [2] were nonlinear in spawning biomass (Fig. 1) and nonlinear fits in spawning biomass were better than linear ones ($p < 10^{-4}$ for management model and $p < 10^{-5}$ for research model, Table 3). The nonlinearity indicates that reproductive success in sardine depends on spawning biomass because constant reproductive success would give a linear fit. Thus, there is a discrepancy between results obtained using our models for log recruitment [1] and log reproductive success [2]. We suggest that the model for log recruitment had greater statistical power and was able to detect the influence of spawning biomass on sardine reproductive success because it was more robust to measurement error. As explained in Hilborn and Walters (1992) and Walters and Ludwig (1981), measurement error is less of a problem in stock-recruitment analysis when there is a lot of contrast in the data and the resulting regression line is steep. Plots of log sardine recruitment data against spawning biomass (Fig. 1) are steep below spawning biomass levels of 1 million t. In

Table 5. Summary statistics for management models [2] fit to log reproductive success data for sardine.

Parameter	Estimate	Standard error	t value	p value
Management model with temperature only				
$\ln(R/S) = \alpha + \beta T$				
α	−16.45	6.077	−2.71	0.011
β_1 (temperature)	1.025	0.358	2.86	0.007
(R^2 = 20%, Var(ϵ) = 0.87)				
Management model with temperature and spawning biomass				
$\ln(R/S) = \alpha + \beta_1 T + \beta_2 S$				
α	−15.12	5.99	−2.53	0.017
β_1 (temperature)	0.961	0.352	2.73	0.010
β_2 (spawning biomass)	−0.0002331	0.0001441	−1.62	0.116
(R^2 = 27%, Var(ϵ) = 0.83)				

Note: The best model was linear in SST (spawning biomass was not significant) but results for models with both spawning biomass and SST are also given. t-tests and p values were used to test the null hypothesis that a true parameter value was equal to zero. There were 34 observations.

Fig. 3. Predicted median recruitment for sardine at 16.5°C from research and management models for log recruitment ln(R) and log reproductive success ln(R/S).

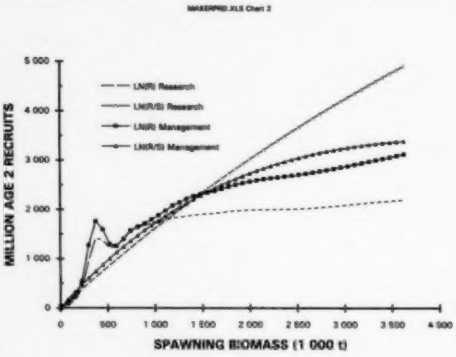
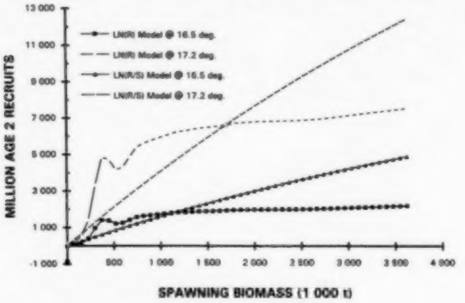


Fig. 4. Predicted median recruitment for sardine from research models for log recruitment ln(R) and log reproductive success ln(R/S). Predicted values are for two values of mean five season SST (16.5 and 17.2°C).



contrast, a plot of log reproductive success for sardine against spawning biomass after adjustment for temperature effects was shallow across the range of spawning biomass levels. The steeper slope in the plot of log recruitment against spawning biomass for sardine is less obscured by measurement error and probably results in greater statistical power.

Serial correlation was apparent in results from most of our models (Fig. 5). Residuals were usually slightly skewed (Fig. 5). Serial correlation in residuals may be due to environmental factors that affect sardine recruitment (Murphy 1966) but were not included in our models.

To gauge effects of serial correlation in residuals, we fit the management model for log reproductive success (model [2]) to spawning biomass and mean three season SST by time series methods (moving average and autoregressive errors at lag 1, Greene 1990). p values from time series models were smaller than or equal to p values from the simple linear regression, indicating that serial correlation did not affect our conclusions. Time series models were useful for gauging effects of serial correlation but were not completely appropriate for our work because sardine spawner-recruit data were interrupted during the 1964–1985 seasons (Table 1). Serial correlation was also included in simulation analyses (see Appendix).

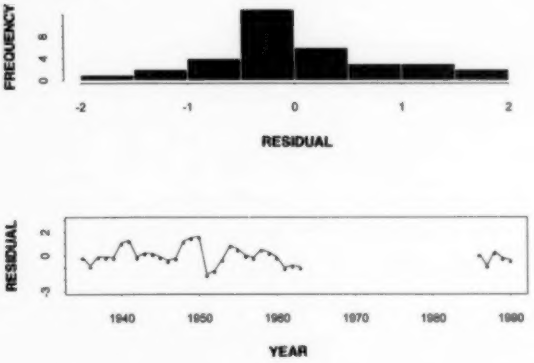
Simulation analyses using the management model for log reproductive success indicate that the parameter for spawning biomass was underestimated by about 23% and that the standard errors for the intercept and temperature parameters were overestimated by about 21%. p values for t-tests with standard deviations estimated by simulation (Table 6) were nearly the same as p values from original tests (Table 5) so conclusions were not affected. Statistical power was evaluated for an alpha level (critical p value used to reject null hypotheses) of 0.05 and assuming parameter values in Table 5. Estimates of the statistical power of model [2] were high (Table 5), but this was due partly to the tendency for the linear regression procedure

Table 6. Results from simulation analyses.

Parameter	Mean from simulations	Bias	% bias	Power
Intercept				
α	−15.2	−0.0343	0	0.71
SD	7.26	1.27	21	
(t-test = −2.52, p value = 0.07)				
Temperature (SST)				
β_1	0.966	0.00466	0	0.76
SD	0.428	0.0756	21	
(t-test = 2.73, p value = 0.05)				
Spawning biomass				
β_2	−0.000288	−0.0000547	−23	0.65
SD	0.000140	0.00000375	−3	
(t-test = −1.62, p value = 0.12)				

Note: Bias is the difference between the value from simulation analyses and the original value (Table 5). Percent bias is bias divided by the original value. p values are for two-sided t-tests on 31 df calculated from the original parameter estimates and standard deviations estimated by simulation. Power is the proportion of simulation runs in which the parameter estimates were statistically significant at the $p = 0.05$ level.

Fig. 5. Time series plot (top panel) and frequency distribution (bottom panel) for standardized residuals from the preferred research model [1] for log sardine recruitment.



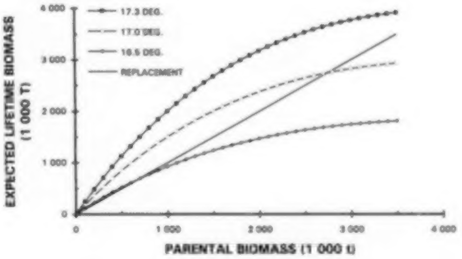
to underestimate standard errors and p values so that the null hypothesis was rejected too often.

Expected lifetime spawning biomass

Stock recruitment plots for sardine and other multiple spawning fish are more easily interpreted if expected lifetime spawning biomass of recruits is calculated and the replacement level of recruitment is defined (Murphy 1966). Lifetime spawning biomass is the spawning biomass of a cohort summed over each year of its life starting when the cohort first reaches sexual maturity. To calculate expected lifetime spawning biomass (E_S), we used

$$[3] \quad E_s = \sum_{a=2}^{20} w_a R e^{-M(a-2)}$$

Fig. 6. Expected lifetime spawning biomass for recruits at mean three season SST levels of 16.3, 17.0, and 17.9°C from the management model [2] for log reproductive success.



where R is predicted recruitment (number of age 2 sardine) from the Ricker management model [2] for a range of spawning biomass (sardine age 2 and older) and mean three season SST levels (Table 5). We used the Ricker management model because it was intermediate among the alternatives and convenient mathematically. The parameter a is age in years, M is the natural mortality rate (0.4/year) for sardine, and the w_a are weights at age from Murphy (1966, Table 16). Recruitment (R) from [3] was corrected for bias because of log transformation (Beauchamp and Olson 1973; Bradu and Mundlak 1970).

The theoretical equilibrium population size for sardine is where spawning biomass and expected lifetime spawning biomass for sardine are the same (i.e., where expected lifetime spawning biomass crosses the replacement line in Fig. 6). Results for temperatures at the first quartile, mean, and third quartile of mean three season SST levels (16.5, 17.3, and 17.0°C) are summarized below (weights in thousand t).

Mean three season SST (°C)	Equilibrium spawning biomass	Maximum sustained yield (MSY)	Spawning biomass at MSY (B_{MSY})	F_{MSY} (%)
16.5	700	9	274	0.04
17.0	2700	156	1272	0.16
17.3	>4000	346	1819	0.26

Predicted equilibrium biomass and MSY for sardine are much lower when environmental factors are less favorable (SST colder) and much higher when environmental factors are more favorable (SST warmer). MSY for sardine is less than or equal to zero ($E_s \le S$ for all S) at all spawning biomass levels when mean three season SST falls below 16.3°C (<3% of all years according to temperature records for 1921–1990). These results are sensitive to choice of models and data, imprecisely estimated recruitment parameters, assumptions about weight at age, and age at recruitment (which may themselves vary with environmental factors) and cannot be regarded as definite or precise. They do indicate, however, that substantial harvests of sardine may be available when environmental factors are favorable (mean three season SST at least average) but little or no harvest may be available when environmental factors are not favorable.

Discussion

Statistical relationships between reproduction of sardine and SST have stood the test of time and appear reliable. According to Marr (1960), hypotheses linking warm water and good sardine recruitment date back to at least 1939. In the same paper Marr (1960) described a positive correlation between a recruitment index for sardine and mean SST at Scripps Pier. Our results clearly indicate that recruitment of sardine to habitat off northern Baja California and the Southern California Bight is dependent on spawning biomass and environmental factors.

Exploratory analyses and modeling indicate that both recruitment and reproductive success are associated with temperature, while cohorts are 1–2 years old. This result was surprising because environmental factors during early life-history stages are generally thought to be most important and because recruitment of Pacific sardine appears related to future environmental conditions. Our explanation involves a hypothesis about the stock structure and availability of Pacific sardine to the fishery.

We hypothesize that Pacific sardine along the west coast of the U.S. and Mexico do not mix randomly in every year. Lack of mixing may be due to discontinuities in habitat or substocks that don't mix completely in each year. Sardine from different locations in U.S. and Mexican waters do not differ genetically based on allozyme data but do show distinct differences in size at age (Hedgecock et al. 1989) that indicate incomplete mixing. Although not conclusive, data (reviewed by Radovich 1982) indicate that sardine in the Gulf of California, southern Baja California, and northern Baja California to the Southern California Bight are distinct. Thus, our hypothesis about the distribution of Pacific sardine is consistent with available data.

The distribution of pelagic fish along the west coast of North America is affected by water temperature (Brodeur and Pearcy 1986; Pearcy and Schoener 1987; Methot 1989). Warm temperatures tend to shift the distribution of pelagic fish to the north and the effect may be most pronounced for the oldest and largest fish (Methot 1989; Smith et al. 1990). Thus, warm SST would increase the availability of sardine from southern habitats to the northern fishery where most of the data used in this study were collected.

VPA recruitment estimates are, essentially, the total number of fish taken by the fishery during the life of a cohort with a correction for natural mortality and an assumption about abundance at the oldest age (Pope 1972). Thus, warmer temperatures that increase availability of sardine from southern habitats to the fishery in the north would also increase the number of fish taken by the fishery and VPA recruitment estimates. The mechanism would be essentially the same for recruitment estimates from other stock assessment models such as CANSAR. Our hypothesis does not preclude the possibility of environmental effects on early life-history stages.

The situation with sardine may not be unique. Recruitment estimates from other nonhomogeneous stocks (MacCall 1986) may also be affected by environmental variables that affect distribution patterns rather than survival of early life-history stages. For example, Myers et al. (1993) found significant relationships between Atlantic cod (*Gadus morhua*) recruitment estimates (from both VPA and young fish surveys) and salinity conditions experienced by juveniles in a situation where movement between management areas was likely.

The mechanism associated with environmental effects is uncertain, but we can use our models to demonstrate and quantify effects of temperature on recruitment and population dynamics of Pacific sardine and resolve some of the controversy about whether environmental factors and fishing were responsible for the collapse of the historic sardine fishery and recent period of recovery. During 1935–1944, mean five season SST were slightly warmer than average (17.1°C), environmental conditions were favorable, and sardine biomass and recruitment were high (Table 1). Adverse environmental conditions, indicated by cold temperatures, prevailed during the mid-1940s to late 1970s. Environmental conditions during the cold period were probably not good enough to produce good reproductive success with the exception of the warm El Niño of 1958 and 1959. The most unfavorable period was 1949–1956 when the mean five season SST was 16.5°C, sardine biomass was low, and median reproductive success probably fell short of the replacement level. Mean five season SST were warm after 1977 and increased to 17.5°C during 1985–1990 while the Pacific sardine population experienced good reproductive success. Thus, it appears that sardine abundance would have declined by late 1970s because of environmental factors only and that the stock rebounded recently in response to environmental conditions.

Historical trends in abundance of sardine were undoubtedly affected by high fishing mortality rates during the period of cold water when biomass declined and by low fishing mortality rates during the period of warm water when the sardine stock began to recover. Mean three season

SST during 1949–1956 was 16.4°C and, according to our management model, the sustainable exploitation rate for sardine was about less than 4% per year. Actual exploitation rates, in contrast, ranged from about 10 to 45% per year (Barnes et al. 1992). In addition to reducing spawning biomass levels, overfishing eliminated older age groups and left the stock more vulnerable to short-term variation in reproductive success (Murphy 1966).

During recent years, sardine exploitation rates have increased as fisheries in the U.S. and Mexico have re-established themselves (exploitation during 1991 may have been as high as 30% per year, Deriso 1993). Current fishing levels may be large enough to impede recovery of the sardine stock, particularly if environmental conditions associated with cool water temperatures prevail. Managers should consider basing annual quota levels and target exploitation rates on recent SST (or other environmental variable) as well as biomass levels.

It is likely that productivity of the sardine resource varies over periods of decades. Baumgartner et al. (1992) found evidence of approximately 60 year cycles in a sedimentary record of sardine abundance. Long-term fluctuations in environmental factors related to ocean temperature (Trenberth 1990) and sensitivity of sardine stock-recruitment relationships to environmental factors related to temperature are a plausible mechanism for long-term cycles in sardine productivity. In the event of a prolonged cold period, little or no sardine harvest may be sustainable.

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Appendix

The sardine fishery and spawner-recruit data for the 1935–1990 fishing seasons were simulated using observed three season SST and catch data (Table 1). For simplicity in the simulation model, recruitment to the fishery and spawning stock occurred at the beginning of each fishing season when sardine were 2 years old (round weight 100 g) and catches were subtracted from the stock biomass at mid-season (Pope 1972). Instantaneous rates for natural mortality (M = 0.4/year) and growth (G = 0.14/year) for sardine age 2+ were assumed constant over time (Zhang and Sullivan 1988). The instantaneous rate for growth (G) was estimated with a linearized growth model (Jacobson et al. 1994) using mean weight at age data from three sources (Murphy 1966, Tables 9 and 15; Deriso 1993, Table 1). Younger age groups were given greater weight in the linearized growth model so that the growth rate at the youngest ages, which is most important in terms of biomass, would be accurately estimated.

Sardine recruitment in the simulation model was calculated using spawning biomass, mean three season SST, and the Ricker management model [2] for log reproductive success which was fit by linear regression (Table 5). Simulated recruitment and spawning biomass data in the model included independent log normal measurement errors.

The lag 1 covariance of residuals from our regression model was 0.3. To mimic this pattern, random numbers used in simulations to simulate recruitment process errors were calculated $\zeta_t = (\epsilon_{t-1} + \epsilon_t)/\sqrt{2}$ where ϵ_t is drawn from the normal distribution with mean zero and unit variance. The series ζ_t has unit variance and a lag 1 autocorrelation of 0.5.

Results for sardine (Deriso 1993) and other stocks (Prager and MacCall 1988) indicate that the coefficient of variation for measurement error in our sardine spawning biomass and recruitment data was probably less than 30%. An arithmetic scale CV of 30% corresponds to a log scale variance of $\ln(0.3^2 + 1) = 0.086$ (Jacobson et al. 1995) and log scale standard deviation of 0.29 for measurement errors in simulated sardine recruitment and spawning biomass data.

We used the variance of residuals from our regression model ($\mathrm{Var}(\epsilon) = 0.83$, Table 5) to specify process and measurement error for sardine recruitment in simulations. The residual variance estimates error from all sources, including process error, measurement error, and model misspecification. Thus, we set the log scale variance for process error to 0.69 so that the average variance of residuals from regression analyses in the simulations was 0.83. Most of the variance in simulated recruitment data around values predicted by the spawner-recruit model (0.69/0.83 = 83%) was due to process error. The simulation model was run 10 000 times and, at the end of each run, the recruitment model [2] was fit to simulated data for the 1935–1963 and 1986–1990 fishing seasons ($N = 34$) by linear regression.

We compared the parameters values used in the simulations to the mean of the simulated estimates to evaluate bias (measured as the difference between the mean of simulated estimates and the original value, Efron 1982). We then compared the standard errors for our original parameter estimates (Table 5) to the standard deviation of the 10 000 simulated parameter estimates to determine if the p values from our statistical tests were misleading. We would conclude, for example, that the p value for spawning biomass in Table 5 was too small if the standard deviation of the slope parameter for spawning biomass in the simulations was much larger than the corresponding standard error in Table 5. For further comparison with values in Table 5, we calculated p values for t-tests on each parameter using the standard deviation of simulated parameter estimates. Finally, we calculated the proportion of instances in which the simulated parameter estimates were statistically significant at the $p = 0.05$ level as a measure of the statistical power of our analysis (Peterman 1990). Statistical power was the probability of obtaining significant parameter estimates for spawning biomass and SST from our data given that recruitment depended on both variables as assumed in our model.

Impacts of zebra mussel on walleye (*Stizostedion vitreum*) reproduction in western Lake Erie

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Abstract: A walleye spawning shoal (Sunken Chicken Reef) in western Lake Erie was sampled during the spring of 1990 and 1991 to assess the abundance of the zebra mussel *Dreissena polymorpha* and to determine its impact on walleye reproduction. Mussel abundance ranged from 76 500/m^2 at 1.5 m depth to 344 300/m^2 at 4.5 m depth at the shoal (1990). Despite the almost total coverage of spawning beds walleye continued to spawn at the shoal, with no apparent effect of the mussels on egg deposition, egg viability, and interstitial dissolved oxygen. Although preinvasion data for walleye egg deposition are not comparable to data from this study, deposition appears adequate based on good indices of recruitment of young-of-the-year walleye derived from resource agency trawling during 1990 and 1991. Thus there appears to have been no adverse impacts of zebra mussel on walleye reproduction in western Lake Erie during 1990 and 1991.

Résumé : Nous avons échantillonné un haut fond servant de frayère au doré jaune dans l'ouest du lac Érié (récif Sunken Chicken) pendant les printemps de 1990 et 1991 pour évaluer l'abondance de la moule zébrée, *Dreissena polymorpha*, et déterminer son impact sur la reproduction du doré. L'abondance des moules se situait entre 76 500/m^2 à une profondeur de 1,5 m et 344 300/m^2 à une profondeur de 4,5 m sur le haut fond (1990). Bien que les frayères aient été presque entièrement couvertes par les moules, les dorés ont continué à y pondre, sans que les moules aient un effet apparent sur le dépôt des oeufs, leur viabilité ni l'oxygène dissous interstitiel. Bien que les données recueillies sur la ponte du doré antérieurement à l'invasion par les moules ne soient pas comparables aux données de notre étude, la ponte semble adéquate si l'on en croit les bons indices de recrutement des jeunes de l'année obtenus grâce aux chalutages effectués en 1990 et 1991 par les organismes de gestion des ressources. Il semble donc que la moule zébrée n'ait pas eu d'effet négatif sur la reproduction du doré jaune dans l'ouest du lac Érié en 1990 et 1991.

[Traduit par la Rédaction]

Introduction

Zebra mussel were first observed in Lake Erie in 1988 and by fall 1989 had been reported from all parts of the lake. The zebra mussel's ability to colonize any hard substrate was of concern to the continued use of shallow rocky substrates by spawning fish. In western Lake Erie, walleye (*Stizostedion vitreum vitreum*) spawn on numerous offshore reefs (Regier et al. 1969), comprised primarily of bedrock (Herdendorf and Braidech 1972), that range in depth from 1.8 to 4.6 m (Keller and Manz 1963; Baker 1967). By 1989, diver observations indicated that most available hard substrate on some reefs had been colonized at densities up to 20 000/m^2 suggesting major effects on reef use by spawning walleye. Walleye have used some of these spawning shoals since the 1800s (Goodyear et al. 1982) and, given their ability to home (see Colby et al. 1979), it was not known what effect such a major and abrupt change to spawning habitat would have on recruitment success. Mussels appear to have almost doubled water transparency (Leach 1992) potentially altering the behaviour of the negatively phototaxic walleye (Ryder 1977). Furthermore, zebra mussel may affect the viability of walleye eggs laid on heavily colonized spawning beds by fouling the reefs with fecal material causing low dissolved oxygen levels which are lethal to walleye (Siefert and Spoor 1974).

The purpose of this study was to quantify the abundance of zebra mussel at an historically important walleye spawning shoal in western Lake Erie and to determine the effects of the mussels on walleye reproduction.

Methods

Study area

Sampling occurred at Sunken Chicken Reef 0.5 km south of Little Chicken Island, which is part of the Chicken Island complex located in the western basin of Lake Erie

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(lat. 41°46′16″, long. 82°47′26″). The shoal, which is similar to others in the region (Herdendorf and Braidech 1972), is a bedrock outcropping about 800 × 300 m at the 2-m contour. Rubble, ranging in size from pebbles to boulders more than 1 m in diameter, is randomly distributed over the reef. The lake bottom surrounding Sunken Chicken Reef is relatively flat and comprised of sand and mud.

Time of spawning

Time of spawning was determined from the catch per unit effort of ripe females in overnight gillnet sets. Each gillnet consisted of seven panels with different mesh sizes (83-, 89-, 102-, and 108-mm stretched measure). Each panel was 32 m long, so each gang was 224 m in length. Gillnets were set at two adjacent walleye spawning shoals, Little Chicken and Hen Islands. In 1990, nets were fished from April 9 to April 23 and in 1991 from April 6 to April 20.

Egg collections

An airlift sampler modified from Mackey (1972) was used by SCUBA divers to collect walleye eggs. A 1-mm mesh nitex bag (30 × 30 cm) was securely fitted to the outlet of the airlift for each individual sample. Although not calibrated, sufficient air was fed to the airlift such that small cobbles could be lifted thus ensuring complete removal of eggs from the substrate. Each sample consisted of the eggs (including chorions) removed from within a 0.5 × 0.5 m quadrat placed at random on the bottom.

Three sets of samples were collected during 1990. Collections on April 19 were intended to assess the effects of water depth, irrespective of numbers of mussels, on egg deposition, and viability. Six samples were collected at 1.5 and 3.0 m and three samples at 4.5 m. Samples were taken at the center and both ends of a 25-m floating transect line. On April 20 quadruplicate samples were taken at 3.0 m from each of three zones subjectively chosen and evaluated by divers as having light (<25%), moderate (25–75%), or heavy (>75%) coverage by zebra mussel to assess the effects of zebra mussel on rate of deposition, hatching, fry deformities, and length at hatch. Light and moderate coverage of the reef by mussels appeared to be the result of ice scour, as mussels in these areas were generally only found in small depressions in bedrock. Moreover there was an abrupt transition around the perimeter of these areas from low coverage of mussels to complete coverage by mussels with an intervening zone containing zebra mussel shell fragments.

Collections made on May 3, 1990, were primarily intended to assess the null hypothesis that walleye spawn indiscriminately of the presence of mussels taking into account the possible dispersive effects of wind-driven currents on egg distribution and the potentially greater retention of walleye eggs by mussel-encrusted over mussel-free areas. As all shoals in the western basin of Lake Erie had been colonized it was not possible to find a "control" shoal in the conventional sense. Instead an area approximately 56 × 128 m at 2–3 m depth that was ice scoured and mussel-free was identified at Sunken Chicken Reef for use as a control. Quadruplicate samples for eggs were taken over the ice-scoured area and compared with samples from transects that ran from the perimeter of the ice-scoured area in southwesterly (prevailing wind direction), southeasterly, northeasterly, and northwesterly directions over mussel encrusted areas. Single airlift samples were taken at distances of 7.6, 15.2, 30.4, and 45.6 m from the perimeter of the ice-scoured area to determine walleye egg densities. Eggs collected were also used to examine the effects of zebra mussel coverage on hatching, length at hatching, and fry deformity rate.

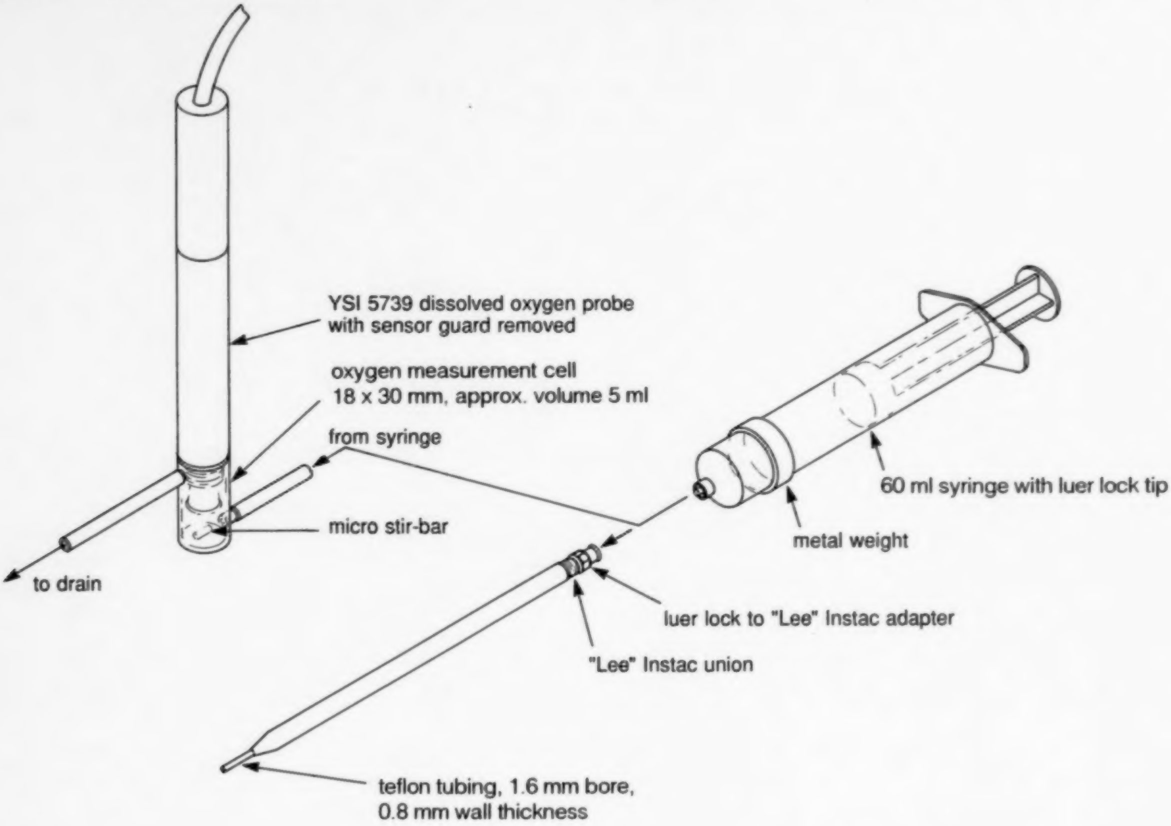
A more intensive survey was conducted during 1991 to repeat and confirm sampling done in 1990 to assess whether walleye spawn indiscriminantly. On May 9 duplicate quadrat (0.5 × 0.5 m) samples were taken at distances of 10, 20, 27.7, 35.4, 50.8, and 66.2 m from the centre of the shoal as in 1990 along eight transects spaced 45° apart starting from north. Mussel abundance for each pair of quadrat samples was qualitatively estimated by a diver and a single cobble collected to estimate density. Using the subjective measure of mussel abundance, an ice-scoured area having less than 25% coverage and approximately 30 × 40 m was identified where control walleye egg number was estimated. Because of the apparent movement of juvenile and adult mussels that had taken place between the spring and the fall of 1990 and the pattern of ice-scour during the winter of 1990–1991 the exact location and size of the ice-scour area used in 1991 was not the same as in 1990.

Nitex sample bags brought to the surface were immersed in surface water at ambient temperature until their contents could be sorted, usually within 2 h. During 1990, eggs were removed from the bags and chorions and opaque dead eggs stored in 10% buffered formaldehyde solution while live translucent eggs were stored live in 250-mL glass jars filled with water (4°C). Staging of live eggs was usually within 96 h with periodic exchanges of water to maintain dissolved oxygen levels. Eggs collected in 1991 were stored in Stockard's Solution (85% water, 5% glycerol, 5% acetic acid, 5% formaldehyde solution).

Total number of eggs, not including chorions, were used to calculate viability and survival to various stages of development. Chorions were not included because there was no way of knowing whether they were the result of predation on viable eggs or mortality of nonviable eggs. Given the difficulty in differentiating dead unfertilized from dead fertilized eggs at early stages of embryogenesis (Olson 1966), no effort was made to determine percent fertilization; hence, equal fertilization rates were assumed. Consequently, numbers of dead eggs include unfertilized eggs as well as dead fertilized eggs. As there was no reason to suspect differential fertilization success associated with the presence of zebra mussel, especially since fertilization occurs above the substrate, the assumption of equal rates of fertilization seemed reasonable.

Stage of development of live and (or) preserved (with Stockard's solution) eggs was determined with reflected light using the criteria and classification scheme of McElman and Balon (1979). By dividing the degree days required to reach individual stages of development from McElman and Balon (1979) by either 7°C in the case of the April 19 (1990) collections or 12°C in the case of the May 3 (1990) collections the incubation period in days to reach the stage was calculated. The incubation period required to reach

Fig. 1. Details of syringe and needle used to sample water from interstitial spaces and modification of dissolved oxygen probe with oxygen measurement cell.


YSI 5739 dissolved oxygen probe with sensor guard removed
oxygen measurement cell 18 x 30 mm, approx. volume 5 ml
from syringe
micro stir-bar
to drain
60 ml syringe with luer lock tip
metal weight
luer lock to "Lee" Instac adapter
"Lee" Instac union
teflon tubing, 1.6 mm bore, 0.8 mm wall thickness

the most advanced stage in the April 19 collections was then used to back-calculate the approximate date of first spawning. Similarly the incubation period required to reach the least advanced stage in the May 3 collection was used to back-calculate the approximate date of last spawning. After staging, all live eggs were reared to hatching in individual 13-cm plastic strainers held in horizontal raceways at an average temperature of 9.7°C. Mortality and hatching were checked daily, with hatched fry placed immediately into 10% buffered formalin. Hatched fry were measured to 0.1 mm using an ocular micrometer, and checked for terata, especially those associated with low dissolved oxygen (Alderdice et al. 1958; Garside 1959).

Quantification of zebra mussels

Zebra mussel coverage was estimated from a combination of subjective diver estimates of degree of colonization (April 19–20 and May 3, 1990) and objective measures of density of colonization of mussels (April 19–20, 1990 and May 9, 1991). Because of the physical impracticality of divers quantitatively removing small quadrat samples of mussels from bedrock, individual 10–20 cm cobbles located in or adjacent to egg sampling quadrats were returned to the surface to determine density. Only those cobbles having percent coverage similar to adjacent bedrock as judged by divers were used for density measurements. Subjective estimates of coverage made by divers were compared with objective measures using correlation analysis.

All live mussels within either a 5 × 5 cm quadrat in 1990 or three to four 2 × 2 cm quadrats in 1991 were removed from individual cobbles and counted and measured to the nearest millimetre to determine density (no./m^2). Length–frequency histograms were constructed from these data.

Dissolved oxygen measurements

In 1990, divers sampled water from the interstitial spaces of cobble deposits at 1.5-, 3.0-, and 4.5-m depths using a 60-mL polypropylene syringe fitted with a 254 × 2 mm (ID) teflon tube (Fig. 1). All void spaces in the barrel and plunger of the syringe (<2 mL total volume) were flooded with surface water prior to water collection when the teflon tube was inserted vertically to its full length into the interstitial space. Immediately after divers returned samples to the surface, dissolved oxygen was measured with a YSI (model 54) oxygen meter fitted with a YSI oxygen sensing probe (model 5739). A 5-mL (internal volume) polystyrene oxygen measuring cell (25 × 40 mm, overall dimensions), with 1-mm (ID) inlet and outlet ports and containing a microspinbar, was screwed onto the probe in place of the sensor guard (Fig. 1). Holding the probe and measurement cell at a 45° angle (from vertical), the 60-mL syringe was attached with plastic tubing to the inlet of the dissolved oxygen measuring cell. The syringe contents were then continuously discharged upwards through the cell to the outlet discharging all air until 30 mL remained in the syringe. The probe and cell were then vertically oriented on a stirring plate and the lowest dissolved oxygen reading observed was recorded. Prior to its use each day, the dissolved oxygen meter was calibrated in air using the wet towel method. Water samples for dissolved oxygen were collected primarily from Sunken Chicken Reef but also along the shoreline of Little Chicken Island.

Environmental data

During 1990, surface temperature was recorded using a mercury thermometer at Little Chicken Island during each gill net set. In 1991 surface temperature was obtained using a temperature logger located adjacent to Little Chicken Island. Daily prevailing wind direction and speed were derived from anemographs obtained from the Atmospheric Environment Service of Environment Canada for a wind anemometer located in Pt. Pelee National Park, approximately 30 km from the study site.

Statistics

Means were compared using either one-way ANOVA or Students *t*-test at $p \leq 0.05$. Percent data (e.g., mortality, survival) were square root (arcsin) transformed prior to testing. Where count data were heteroscedastic they were logarithmically (common) transformed prior to testing or tested using the Mann–Whitney *U*-test. Correlations between indices of mussel abundance were calculated using Spearman's Rank test of correlation.

Results

Zebra mussel abundance

Estimated mean numbers of mussels on Sunken Chicken Reef on April 19 and 20, 1990, increased with depth (F = 4.82, p = 0.019) ranging from 76 500/m^2 at 1.5 m to 344 500/m^2 at 4.5 m (Table 1). Over 90% had a shell length of less than 10 mm. By May 9, 1991, this difference was not significant even though numbers showed some variation with depth, ranging from 80 000/m^2 at depths less than 2 m to 106 750/m^2 at depths greater than 4 m. Moreover numbers of zebra mussel in 1991 were reduced over densities in 1990 with significant declines of 42% at depths of 2–4 m and 69% at depths greater than 4 m. As in 1990, over 90% of the mussels had a shell length of less than 10 mm. Reduction in numbers in this same size range was primarily responsible for the changes in density from 1990 to 1991 (Fig. 2). Mussel density was unrelated to the type of hard substrate at Sunken Chicken Reef.

Table 1. Estimated mean coverage and density of zebra mussels at various depths (m) at Sunken Chicken Reef, 1990 and 1991.

	Depth (depth range, m)		
Parameter	1.5 (<2)	3.0 (2–4)	4.5 (>4)
% Coverage			
1990			
Mean	23	54	87
SD	24	33	6
N	6	15	3
1991			
Mean	63	74	84
SD	29	35	36
N	17	26	5
Density (no./m^2)			
1990			
Mean	76 500	135 000	344 300
SD	80 300	76 400	141 600
N	6	15	3
1991			
Mean	79 900	77 500	106 800
SD	61 700	38 900	43 800
N	16	24	3

Diver estimates of mean coverage by zebra mussel of substrate during 1990 ranged from 23% at 1.5 m to 87% at 4.5 m with significantly (F = 4.82, p = 0.019) less coverage at 1.5 m (Table 1). In contrast during 1991 coverage was independent of depth ranging from 63% at depths less than 2 m to 84% at depths greater than 4 m (Table 1). Only at depths less than 2 m, was there a substantial increase in coverage from 1990 to 1991, probably the result of the reduced ice scour at shallower depths documented by divers during 1991. Coverage was affected by the type of substrate. Percent bedrock was positively correlated (r_s = 0.43, $p < 0.05$) and percent gravel was negatively correlated ($r_s = -0.52$, $p < 0.05$) with zebra mussel coverage at Sunken Chicken Reef on May 9, 1991. Despite some concurrent changes in density and coverage with depth, coverage was not correlated with density in either 1990 or 1991.

Walleye spawning

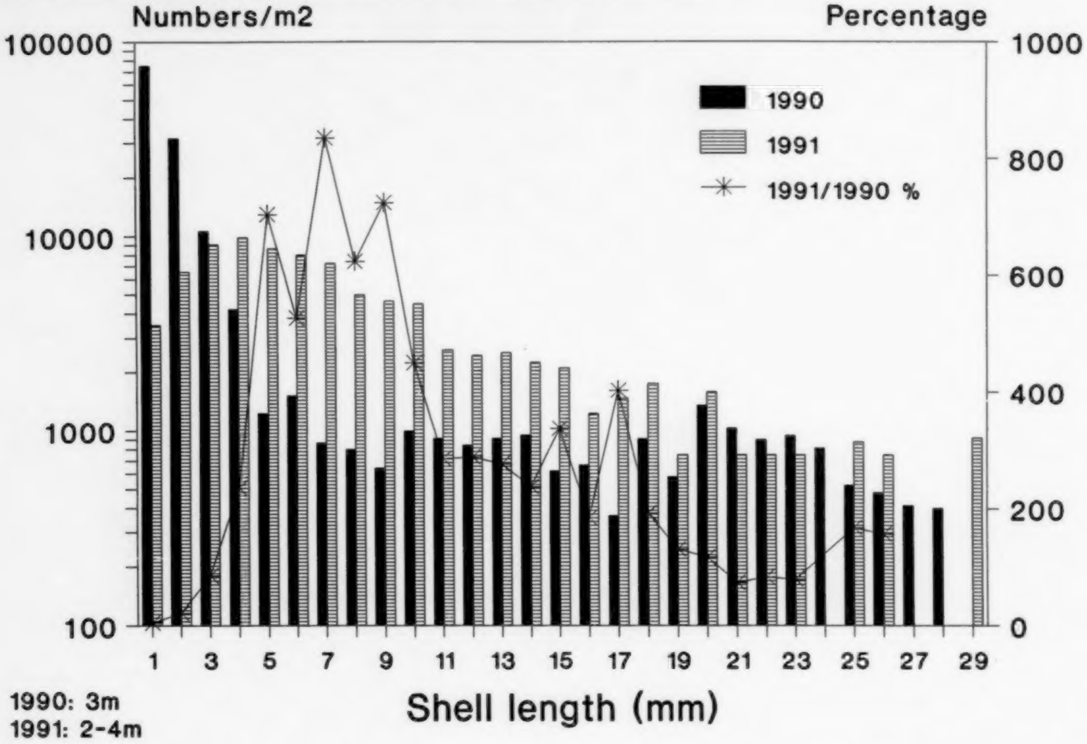
By using egg stages, we calculated that spawning in 1990 spanned approximately April 16 to May 2 with over 50% of the egg-incubation period being reached by May 3. These calculations were in agreement with gill net catches of ripe females that increased sharply on April 17 and were associated with increasing ambient water temperature (Fig. 3).

During 1991, it was calculated from egg stages that spawning began on April 18 and lasted until approximately May 8. As in 1990, initiation of spawning was associated with high numbers of females in gill nets and increasing ambient water temperature (Fig. 3).

Effects of zebra mussel on numbers of eggs collected

Walleye eggs were found at depths from 1.5 to 4.5 m with highest densities at 3.0 m during 1990. Mean egg density

Fig. 2. Length–frequency distributions for zebra mussel collected in 1990 at 3-m depth and 1991 at 2–4 m depth at Sunken Chicken Reef and the relative difference (%) between 1991 and 1990.



(no./m^2 ± SD) at 1.5 m (98.0 ± 65.3) was similar to that at 3.0 m (214.7 ± 148.6), although density at 4.5 m (48.0 ± 41.6) was significantly less (Mann–Whitney $U = 17$, $p = 0.05$) than at 3.0 m.

At 3 m the presence of mussels had no effect ($F = 0.63$, $p = 0.56$) on egg density early in the spawning period (April 20, 1990). Mean egg density (no./m^2 ± SD) for areas having low, medium, or high coverage of zebra mussels was 163.0 ± 96.7, 170.0 ± 145.4, and 296.0 ± 277.2, respectively. In addition, there was no significant relationship between egg and mussel density ($F = 0.68$, $p = 0.43$). Similarly later in the spawning season (May 3), there was no relation between zebra mussel coverage and egg density at 2–3 m ($F = 0.59$, $p = 0.57$). Mean egg densities (no./m^2 ± SD) for areas of low, medium, and high coverage were 99.2 ± 77.2, 51.2 ± 51.6, and 87.2 ± 80.7 respectively.

Effects of location on shoal on numbers of eggs collected

The number of walleye eggs collected was similar in areas with and without zebra mussels during 1990. Although prevailing winds for Sunken Chicken Reef for the period April 16–30 were from the southwest (Table 2), egg densities on an area southwest and windward of an ice-scoured mussel-free area were similar to densities found on the northeast and leeward side of the shoal. Furthermore there were no consistent patterns in egg density with decreasing or increasing distance from the centre of the ice-scoured area that could be related to wind patterns. Finally there were no significant differences in the egg density among the four transects and ice-scoured area (Table 3).

Egg density during 1991 appeared independent of the relative abundance of mussels as well. Although prevailing wind was from the northeast (Table 2), egg density on the reef showed no consistent pattern with decreasing or increasing distance downwind from the ice-scoured area. Furthermore, there were no significant differences in the density of walleye eggs collected from the unscoured areas of the eight transects and the ice-scoured area of the east and southeast transect (Table 3). During 1991, however, the pattern of egg deposition relative to mussel abundance was considerably less random than in 1990. Highest numbers of eggs (22 eggs/m^2) were collected on the ice-scoured area; however, of seven sites sampled on the ice-scoured area, five had no eggs. High numbers of eggs were collected on the south transect immediately downwind of the ice-scoured area. However, samples taken immediately adjacent to the ice-scoured area samples on the same transect had no eggs. It is recognized though that with low numbers of eggs collected in 1991, possibly related to high winds, conclusions are at best tentative.

Effects of zebra mussel on water quality and egg viability

Dissolved oxygen levels amongst cobble substrate encrusted with zebra mussels were still quite high. Mean dissolved

Fig. 3. Catch-per-unit effort (no./overnight gill net set) of ripe female walleye and water temperature adjacent to Little Chicken and Hen Islands during the spawning period in 1990 and 1991.

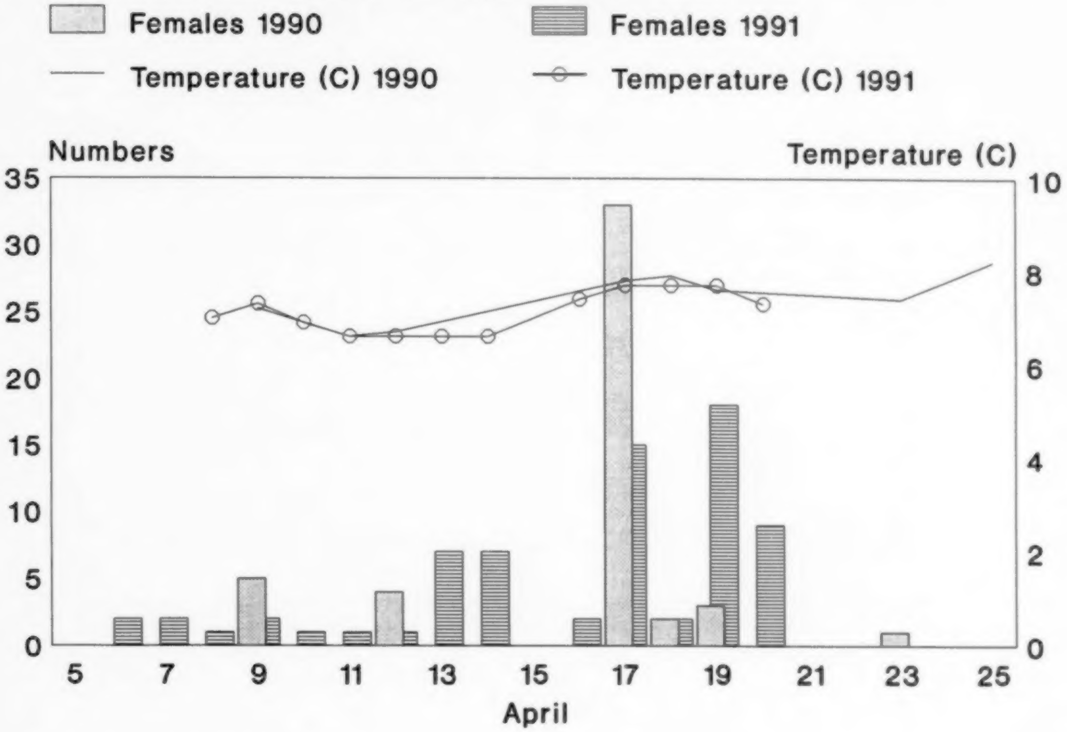


Table 2. Summary of wind data.

Direction	% of spawning period		Average speed (km/h)	
	1990	1991	1990	1991
North	0.8	10.4	6.4	20.9
Northeast	3.6	28.0	13.2	19.4
East	3.6	13.4	7.5	10.0
Southeast	28.6	16.1	11.5	11.7
South	6.7	0.3	10.1	1.7
Southwest	41.4	20.8	12.2	15.1
West	2.5	1.8	5.7	7.2
Northwest	12.8	9.2	15.0	15.7

Note: Data were derived from a wind anemometer located in Pt. Pelee National Park, for the period April 16 to April 30, 1990, and April 17 to April 30, 1991.

Table 3. Mean walleye egg density (no./m^2) per transect from five areas at Sunken Chicken Reef on May 3, 1990 ($N = 4$) and eight areas on May 9, 1991 (the centre transect was the ice-coured area in 1990).

Area	Mean (SD)		
	1990	1991	*N*
Centre ice scour	105.0 (87.9)	5.3 (14.1)	16
North		0 (0)	12
Northeast	86.0 (52.1)	4.3 (6.3)	12
East		1.6 (3.6)	5
Southeast	69.0 (72.1)	2.0 (3.3)	6
South		1.5 (4.2)	8
Southwest	103.0 (115.2)	0.8 (1.7)	10
West		0.7 (1.6)	12
Northwest	43.0 (28.0)	2.2 (1.2)	12

oxygen levels (mg/L ± SD) in heavily encrusted cobble deposits on April 19, 1990, at 1.5, 3.0, and 4.5 m were 10.3 ± 0.8, 10.5 ± 0.4, and 10.9 ± 0.1, respectively, and were greater than 80% saturation.

There was considerable collection variation in egg viability within individual mussel abundance categories. Thus the presence of zebra mussel had no significant effect on either fertilization success or survival of eggs at the time of collection during 1990 (Table 4). Similarly, when eggs collected on April 20 and May 3 were held in the laboratory, percent hatch, percent live hatch, percent deformed, and length at hatch also showed considerable variation

Table 4. Average viability at time of collection, percent hatch, percent live hatch, percent deformities at hatch and length for walleye eggs collected from three levels of mussel colonization on April 20 and May 3 (1990) at Sunken Chicken Reef and reared to hatch in the laboratory at an average temperature of 9.7°C.

		Level of colonization					
		Low		Medium		High	
Parameter	Date	X (SD)	N	X (SD)	N	X (SD)	N
Viability	April 19	95.3 (5.5)	4	96.4 (7.3)	5	96.0 (4.9)	6
	April 20	62.3 (15.5)	4	65.2 (17.5)	4	67.0 (4.4)	4
	May 3	83.9 (19.9)	5	92.4 (7.8)	5	68.7 (24.2)	10
% hatch	April 20	58.3 (14.0)	4	55.4 (14.5)	4	55.4 (15.3)	4
	May 3	72.3 (27.0)	5	67.1 (28.2)	5	41.9 (26.9)	10
% live hatch	April 20	51.0 (15.5)	4	52.4 (10.7)	4	42.3 (15.5)	4
	May 3	63.7 (25.7)	5	67.1 (28.2)	5	37.8 (24.1)	10
% live deformed at hatch	April 20	0	4	2.5 (3.4)	4	2.7 (4.5)	4
	May 3	5.0 (11.2)	5	1.3 (2.8)	5	4.2 (7.3)	10
Length at hatch (mm)	April 20	7.7 (0.7)	4	7.1 (1.0)	4	7.8 (0.3)	4
	May 3	7.4 (0.3)	5	7.6 (0.3)	5	7.6 (0.4)	10

within individual mussel abundance categories. As such, mussel abundance was found to have no effect on eggs held in the laboratory after collection (Table 4).

Discussion

Zebra mussel density at Sunken Chicken Reef during 1990 was among the highest reported for shallow waters in either Europe (Mackie et al. 1989) or North America (Griffiths et al. 1991; Leach 1992). Higher densities have been reported in western Lake Erie (700 000/m^2) but were associated with a water intake (Griffiths et al. 1991).

The increase in density with increasing depth evident in 1990 was similar to the pattern in Polish lakes (Stanczykowska 1964) where density increased from 2 to a maximum at 4 m. At Sunken Chicken Reef density of mussels at shallower depths (<2 m) was affected by ice scour. Between 1990 and 1991 large declines in density occurred at greater depths (>2 m), although density at shallower depths remained relatively constant. Because divers observed more evidence of ice scour at shallower depths in 1990 than 1991, numbers at these depths in 1990 were probably depressed and hence no change in density was observed between 1990 and 1991.

The decline in mussel density between 1990 and 1991 at depths >2 m was largely the result of reduced recruitment as indicated by the length–frequency distributions. This was supported by Leach (1992) who felt that, based on declining rates of inter-annual increases in density of zebra mussel veligers, populations in 1990 were approaching an asymptotic maximum. Declines have also resulted from loss of older zebra mussel individuals at Sunken Chicken Reef based on the length–frequency distributions.

Walleye continued to use Sunken Chicken Reef for spawning in 1990 and 1991 despite the high standing populations of zebra mussel. However, it is not known how spawning activity during these 2 years compared with that documented for the area prior to colonization by mussels. Timmerman (1988), using a suction pump towed over approximately 132 m^2 of bottom at depths of 3–4 m adjacent to Little Chicken Island, collected 72 walleye eggs per tow or 0.55 eggs/m^2 early in the spawning period and 12 eggs/tow (0.09 eggs/m^2) near the end of the spawning period. Egg deposition studies have also been conducted on shoals in the southern portion of Lake Erie's western basin, but they too cannot be compared with our study because of differences in collection methodology. Using a suction pump towed over approximately 264 m^2 of bottom, Busch et al. (1975) over a 10-year period collected walleye eggs that ranged from 100 to 900/tow or from 0.38 to 3.41 eggs/m^2. Relative to egg density in the present study these authors' estimates of egg deposition are very low and probably relate to the low efficiency of the methods they used. Nevertheless, based on recruitment of young-of-the-year (YOY) walleye, numbers of eggs deposited in both 1990 and 1991 were adequate for recruitment and may in fact have exceeded that of the previous 3 years when mussel abundance was lower. The index of abundance of YOY walleye in western Lake Erie for 1990 was 3.5- to 26-fold higher than for the previous 3 years (Ontario Ministry of Natural Resources 1992). This index is probably most affected by the success of open lake spawning stocks as opposed to riverine spawning stocks. Busch et al. (1975) found year class strength of western Lake Erie walleye was related to wind-generated temperature reversals in the lake such that the high YOY abundance that occurred during 1990 probably represents production from open lake shoals, not river spawning stocks. Of the two major riverine spawning stocks in western Lake Erie, the Sandusky and Maumee rivers, the Sandusky River spawning stock has been in decline since the mid-1970s (R. Knight, Columbus, Ohio, Ohio Department of Natural Resources, personal communication).

The foregoing suggests that open lake walleye shoals have not been affected by mussels. On the contrary, presence of mussels and the increase in interstitial space they provide may have increased egg survival and recruitment by preventing egg dislodgement (Busch et al. 1975) and predation (Wolfert et al. 1975) that are detrimental to survival. Numbers of eggs collected at Sunken Chicken Reef on April 20 (1990) were 1.8-fold higher on substrates with high mussel abundance than substrates with low mussel abundance, although because of considerable variation this difference was not significant.

Depths over which eggs were collected in this study (1.5–4.5 m) were similar to those reported for the northerly (2.1–6.1 m) (Timmerman 1988) and southerly (1.8–4.6 m) (Keller and Manz 1963; Baker 1967) portions of western Lake Erie. The decrease in egg deposition at 4.5 m relative to 3.0 m noted in this study has not been previously documented. Recent observations (D. Wolfert, USFWS, Sandusky, Ohio, personal communication) for the southern portion of western Lake Erie indicate that spawning occurs no deeper than 3.7 m. Apparent egg deposition would most likely be a function of currents and bathymetry with eggs found at greater depths potentially moved there by currents from shallower depths. In any event, the apparent range of depth of egg deposition in this study appears similar to that reported prior to the invasion by zebra mussel. Hence the almost twofold increase in water transparency in western Lake Erie associated with zebra mussel (Leach 1992) has not resulted in any compensatory increase in spawning depth by the negatively phototaxic walleye (Ryder 1977).

Dissolved oxygen, although only sampled over 2 days, was apparently little affected by the presence of zebra mussel to the extent of being lethal to walleye eggs. This lack of any substantial effect on dissolved oxygen does not appear related to reduced metabolic activity of mussels during the cold spring temperatures (Quigley et al. 1992). Divers noticed widespread egestion of pseudofaeces while swimming over mussel beds. The lack of an effect is more likely to be related to the generally shallow nature of the substrate with few interstices, the continuous water exchange by wind-generated currents and the relatively low temperature. Consequently, although numbers of mussels were extremely high on Sunken Chicken Reef, it is unlikely that the measured dissolved oxygen levels represent the full potential for depression of dissolved oxygen by mussels. We expect that dissolved oxygen at spawning shoals in more protected and deeper substrates and at higher temperatures would be more affected by the presence of mussels.

Although the limited water sampling does not provide unequivocal proof that Sunken Chicken Reef did not experience reduced oxygen levels, walleye eggs obtained at the start and end of the spawning period showed no evidence of chronic low oxygen stress. Siefert and Spoor (1974) reported a drop in walleye egg survival at 35% saturation while Oseid and Smith (1971) observed a decline in length-at-hatch when walleye eggs were held at 50% saturation. Neither egg survival or length-at-hatch were affected in eggs collected from areas with zebra mussel, although admittedly the high variation reduced the power of the statistical tests used. Although the deformities at hatch we observed were similar to those produced by low oxygen levels (Garside 1959), they were similar whether the eggs were collected from areas with low or high mussel coverage. In addition the occurrence of deformities was similar in eggs collected at the start and end of the spawning period. Moreover, the proportion of eggs viable at time of collection was similar to the 33–100% reported previously for western Lake Erie (Timmerman 1988). Hence there was no evidence of any overall decline in walleye egg viability associated with colonization by mussels.

In conclusion, despite the dramatic change in spawning habitat associated with zebra mussel, walleye continued to spawn at Sunken Chicken Reef, a representative western Lake Erie spawning shoal. Coupled with the above average index of YOY walleye in assessment netting (Ontario Ministry of Natural Resources 1992) during the same period there appears to have been no adverse effects of mussels on recruitment. It is difficult, however, to extrapolate the results of this study to other walleye shoals in the Great Lakes or inland lakes owing to differences in zebra mussel density, predation, water circulation patterns, and shoal structure. It is even more difficult to extrapolate to other shoal spawning species such as the lake trout because egg size, spawning temperature, and incubation period will also determine the effects of mussels.

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Seasonal variation in water chemistry over a bog-rich fen gradient in Continental Western Canada

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Abstract: Surface and subsurface (0.5, 1.0, and 1.5 m depths) water was sampled weekly in 1989 and biweekly in 1990 during the ice-free season along a bog-rich fen gradient in central Alberta. Acidity–alkalinity were most closely related to peatland type and were the most useful parameters for characterizing peatlands. Potassium, nitrogen, and phosphorus concentrations were more related to season, year, or peatland–year interactions and cannot be used to categorize the bog–fen gradient. Hydrogen ion, ammonium, alkalinity, and corrected conductivity were relatively constant throughout the ice-free season, while total metal ions (Al, Fe, Mn, and Zn), base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), nitrate, and components of phosphorus fluctuated seasonally. Nitrate remained constant with depth in all peatland types, whereas ammonium increased with depth. Relationships of surface water chemistry to pH for all sites showed three patterns: a positive and highly significant correlation with little seasonal variation within peatland types (base cations, alkalinity, and corrected conductivity); less significant correlation with strong seasonal variation within peatland types (N and P); and a general negative and highly significant correlation with some seasonal variation in peatland types (metals and S). Water temperatures increased along the bog-rich fen gradient.

Résumé : Nous avons échantillonné l'eau superficielle et subsuperficielle (0,5, 1,0 et 1,5 m de profondeur) chaque semaine en 1989 et toutes les deux semaines en 1990 pendant la saison libre de glace le long d'un gradient de tourbières allant de la tourbière oligotrophe à la tourbière fortement minérotrophe dans le centre de l'Alberta. L'acidité et l'alcalinité étaient liées surtout au type de tourbière, et constituaient les paramètres les plus utiles pour caractériser ces tourbières. Les concentrations de potassium, d'azote et de phosphore étaient davantage liées à la saison, à l'année ou aux interactions type-année, et ne peuvent servir à établir des catégories dans le gradient oligotrophe–minérotrophe. L'ion hydrogène, l'ammonium, l'alcalinité et la conductivité corrigée étaient relativement constants pendant toute la saison libre de glace, tandis que les ions métalliques totaux (Al, Fe, Mn et Zn), les cations basiques (Ca^{2+}, Mg^{2+}, Na^+, K^+), les nitrates et les composants du phosphore fluctuaient de façon saisonnière. Les nitrates demeuraient constants quelle que soit la profondeur dans tous les types de tourbières, tandis que l'ammonium augmentait avec la profondeur. Les relations entre la chimie de l'eau superficielle et le pH dans tous les sites présentaient trois profils : une corrélation positive et hautement significative avec une faible variation saisonnière au sein des types de tourbières (cations basiques, alcalinité et conductivité corrigée); une corrélation moins significative avec forte variation saisonnière au sein des types de tourbières (N et P); enfin, une corrélation globalement négative et très significative avec une certaine variation saisonnière selon les types de tourbières (métaux et S). La température de l'eau augmentait le long du gradient oligotrophe–minérotrophe.

[Traduit par la Rédaction]

Introduction

Peat-forming ecosystems are an important component of northern landscapes that have developed when plant biomass formed by primary production is not completely decomposed. The formation of peat gradually isolates the surface of the peatland from the influences of the surrounding mineral soils and the peatland becomes increasingly dependent on precipitation for its nutrient and mineral requirements. Peatlands have been classified using

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hydrological criteria as fens (geogenous systems influenced by ground water) or bogs (ombrogenous systems influenced only by precipitation). Based on vegetative criteria, fens have been further classified into *Sphagnum*-dominated poor fens and "brown moss"-dominated rich fens (DuRietz 1949). Later Sjörs (1950, 1952) related acidity and conductivity to this vegetation-based classification of fens. Rich fens have been divided into transitional (= moderate) rich fens and extreme-rich fens (Sjörs 1950, 1952), each correlated with vegetation and chemical characteristics (Vitt and Chee 1990). Based on these works, the concepts of bog, poor fen, and rich fen have become deeply entrenched in the peatland literature (e.g., Wassen et al. 1989), especially that originating from Fennoscandia. Chemical variation along the bog-rich fen gradient has been documented by numerous workers (reviews by Malmer 1986; Gorham et al. 1987; Vitt 1990).

Water chemistry in peatlands is controlled by precipitation chemistry, surface and subsurface water chemistry, plant uptake, cation exchange mechanisms, and the microbial processes of decomposition and mineralization. The relative importance of these factors are the prime control on peatland water chemistry and ultimately of peatland processes and ecosystem function. As water chemistry in peatlands can be easily quantified, it can be a useful indicator of peatland function. The first step in understanding peatland ecosystems is to delimit the temporal and spatial distribution of the chemical components in the water to establish trends and relationships.

In western Canada, base cation chemistry has been used to characterize a variety of peatland types, summarized by Vitt and Chee (1990) and Vitt (1990). More specifically, bogs have been studied by Horton et al. (1979), Vitt and Bayley (1984), Zoltai and Johnson (1985), and Nicholson and Vitt (1990); poor fens by Vitt et al. (1975), Horton et al. (1979), Vitt et al. (1990); rich fens by Slack et al. (1980), Karlin and Bliss (1984), Rochefort and Vitt (1988), and Chee and Vitt (1990). These studies have largely been devoted to describing the spatial patterns of variation in water chemistry between peatland sites, determining the relationships between surface water chemistry and vegetation pattern, and demonstrating relationships between surface water chemistry and peat chemistry or regional precipitation patterns (Zoltai and Johnson 1987; Gignac 1990; Malmer et al. 1992). These studies have clearly shown that peatland type, peat accumulation patterns, and vegetation patterns are strongly related to particular chemical gradients present in surface waters (Chee and Vitt 1990).

Relationships between chemical components of surface waters, usually the four base cations (Ca^{2+}, Mg^{2+}, Na^+, and K^+), corrected conductivity, and pH have been well described by the authors cited above. From these data it is well established that bogs and poor fens have surface waters characterized by little or no alkalinity, pH less than 5.5, and low concentrations of calcium and magnesium, while rich fens have more alkaline waters with the pH higher than 5.5 and relatively high concentrations of calcium and magnesium. However, it is less well understood how these surface water concentrations relate to the subsurface water chemistry.

Solubility of metal ions as a function of pH is well known in agricultural and lake ecosystems; however, it has been less well studied in peatland waters. Several authors have shown that bog peat retains Al and Fe more strongly than Mn (Clymo 1963; Wieder and Lang 1986), but there have been fewer studies of the surface water geochemistry (Urban et al. 1987; Helmer et al. 1990). Seasonal variability of these metal ions is also poorly known in peatland surface waters, particularly in fen waters.

Although much has been written relating cations and acidity to vegetation and the bog–fen gradient, few studies of other elements and ionic components have been reported. For example, concentrations of strong acid anions, alkalinity, and nutrients (NH_4^+, NO_3^-, and phosphorus) have been reported in only a few papers (Hemond 1983; Vitt and Bayley 1984; Wieder 1985; Urban and Eisenreich 1988). It is unclear how surface water nutrients vary along the bog – rich fen gradient, how nutrient concentrations vary with depth, and whether there are annual variations. There are also few studies available that describe chemical characteristics of surface waters or ground waters of individual peatlands over time (Verry and Timmons 1982; Vitt and Bayley 1984; Urban and Eisenreich 1988). This paper addresses the temporal and spatial aspects of peatland biogeochemistry in western Canada.

Objectives

The objectives of this study are to determine for southern boreal continental peatlands in western Canada: (*i*) the patterns in water chemistry present along a vegetational gradient from bog, through poor fen to rich fen and (*ii*) the temporal pattern in water chemistry over a 2-year period (1989–90) for these wetlands. These patterns will then be used to (*iii*) relate variation in the surface water chemistry to that of the subsurface water along these temporal and spatial gradients and (*iv*) quantify the variation found in nutrients (NH_4^+, NO_3^-, P) to those of base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), metals (Al, Mn, Fe, Zn), sulphur, alkalinity, and conductivity. Based on these objectives, we pose the following four questions. (1) What components of the surface water chemistry can be used to corroborate the classification system ranging from bog to rich fen currently based on vegetation and hydrology? (2) Is there significant temporal variation in surface water chemistry? (3) Does subsurface water chemistry differ from that of the surface water? (4) Are spatial and temporal patterns of nutrients different from those of the base cations, metals, and sulphur? The answers to these questions will lead to a better understanding of peatland ecosystem function and improved water sampling strategies for future studies.

Study area

The study area, in central Alberta, is located between 54° and 55°N latitude and about 113°W longitude in the Subhumid Low Ecoclimatic Region (Ecoregions Working Group 1989). The area is characterized by warm summers and cold, snowy winters. Total annual precipitation is about 490 mm, being highest in June and July. The average mean annual temperature is 1.4°C and degree days above 0°C average 2314 for the 30-year period between 1951–1980. The frost-free period is about 120 days long.

Fig. 1. Location of study sites. Sites are (*a*) bog, (*b*) poor fen, (*c*) extreme-rich fen (open), (*d*) moderate-rich fen (forested), and (*e*) moderate-rich fen (open). Peatland distribution has been modified from Vitt (1992) and includes areas where peatland coverage is >50%.

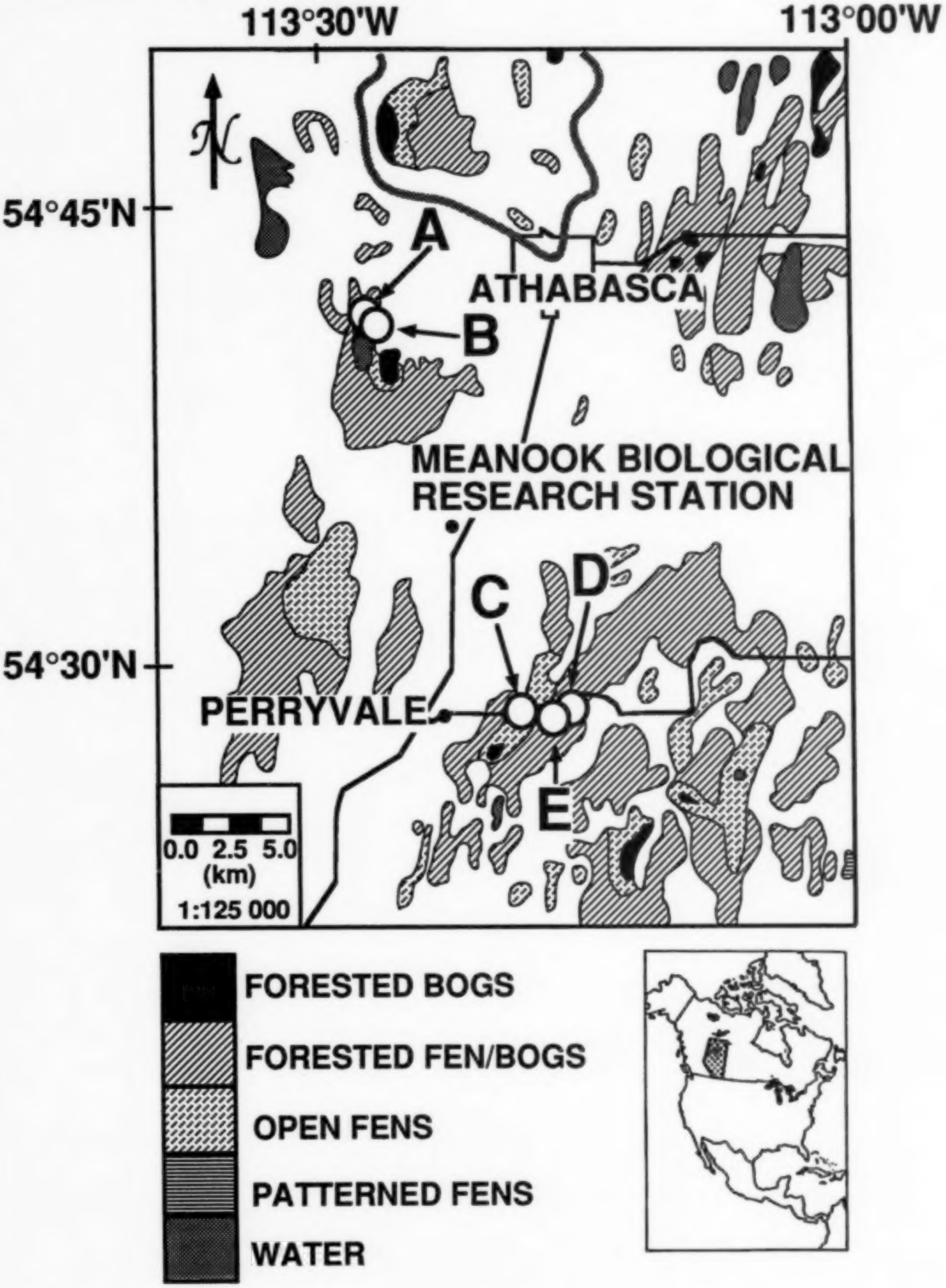

113°30'W
113°00'W
N
54°45'N
A
ATHABASCA
B
MEANOOK BIOLOGICAL RESEARCH STATION
54°30'N
C
D
PERRYVALE
E
0.0 2.5 5.0
(km)
1:125 000
FORESTED BOGS
FORESTED FEN/BOGS
OPEN FENS
PATTERNED FENS
WATER

Table 1. General characteristics and location for the five peatlands.

Peatland type	Site	Lat., long., elev.	Physiography	Dominant vegetation
Bog	Bleak Lake	54°41′N, 113°28′W, 610 m	Forested hummocks	*Picea mariana*, *Sphagnum fuscum*
Poor fen	Bleak Lake	54°41′N, 113°28′W, 610 m	Nonforested lawn	*Carex tenuiflora*, *Sphagnum teres*
Moderate-rich fen (FMRF)	Colinton Creek	54°28′N, 113°17′W, 678 m	Forested hummocks	*Larix laricina*, *Tomenthypnum nitens*
Moderate-rich fen (OMRF)	Colinton Creek	54°28′N, 113°17′W, 678 m	Nonforested carpet	*Carex lasiocarpa*, *Drepanocladus vernicosus*
Extreme-rich fen (ERF)	Perryvale	54°28′N, 113°20′W, 663 m	Nonforested carpet	*Carex lasiocarpa*, *Drepanocladus aduncus*

The upland vegetation is mostly deciduous forest, dominated by *Populus tremuloides*. *Picea glauca* and *Abies balsamea* are uncommon because of high fire frequency. *Populus balsamifera* is of secondary importance, while *Pinus banksiana* is restricted to dry sandy sites. Forested wetlands are dominated by *Picea mariana* and *Larix laricina* and represent about 12% of the study area.

The study area has a gently rolling topography that is underlain by Upper Cretaceous clastic marine and continental deposits of sandstones and shales (Douglas et al. 1976). Surficial sediments consist of sands and gravels, tills, glacio-lacustrine deposits, and scattered aeolian deposits (St. Onge 1972*a*, 1972*b*). Soils are dominantly Grey Luvisols with Eutric and Dystric Brunisols occurring on dry sites. The area is considered to have been glaciated more than once during the Pleistocene (St. Onge 1972*a*), becoming ice-free approximately 11 000 – 12 000 years B.P. (Dyke and Prest 1987).

Methods

Site selection

Based on extensive field surveys and air photo interpretation conducted throughout Alberta, five representative sites were chosen to encompass the broadest possible range of peatland types present in the southern boreal forest (see Vitt and Chee 1990 and Vitt 1992 for definitions and occurrences of peatlands in Alberta). These five sites include an ombrotrophic bog, poor fen, forested moderate-rich fen, open moderate-rich fen, and extreme-rich fen (with some marsh characteristics). Sites were selected within a 20-km radius of Meanook Biological Research Station, 14 km south of Athabasca (Fig. 1), in central Alberta.

Site descriptions

General characteristics and specific locations for the five sites are given in Table 1. A general description of each site follows.

Bog

The bog site is a large, raised, ombrotrophic island within the Bleak Lake peatland complex bordered on the east side by a poor fen water track. The peat surface is raised about 0.3–0.5 m above the associated water track and is composed of large dry hummocks separated by wetter hollows with a height difference. Surface water is not present. The vegetation of the bog is characterized by a sparse tree cover of small *Picea mariana*, with a shrub/herb layer of *Ledum groenlandicum*, *Vaccinium oxycoccos*, *Smilacina trifolia*, and *Rubus chamaemorus*. Isolated hummocks of *Eriophorum vaginatum* are present. The ground layer is dominated by 70% cover of *Sphagnum fuscum* on the hummocks, with smaller amounts of *S. magellanicum* and *S. angustifolium* in the hollows. Several species of the lichen genus *Cladonia*, as well as *Cladina mitis* occur frequently on the drier hummocks. Peat depth is approximately 5 m, with the top 170 cm composed entirely of *Sphagnum*. For this top *Sphagnum* layer, bulk density is about 0.040 g/cm^3, and C/N ratios range from 36 to 58.

Poor fen

This fen lies adjacent to the bog island (Fig. 1) and provides a north–south track for water movement. The peat surface is a lawn containing sporadic low hummocks. The site is wetter than the bog, with surface water present throughout much of the season. This water track is essentially treeless and is characterized by a well-developed shrub layer of *Betula glandulosa*, *Salix discolor*, and *S. pedicellaris*. Also common is the dwarf shrub *Andromeda polifolia* and the herb *Potentilla palustris*. Sedges are abundant, especially *Carex chordorrhiza*, *C. paupercula*, *C. tenuiflora*, and *C. lasiocarpa*. The ground layer is composed of about 70% *Sphagnum* and 30% brown mosses. Dominant are *Sphagnum teres*, *S. angustifolium*, and *Tomenthypnum nitens*. Peat depth is about 4 m, with fairly consistent botanical composition throughout its length. Bulk density ranges from

0.04 to 0.19 g/cm^3 and C/N ratios from 10 to 16 from the top of the profile to the base.

Moderate-rich fen (forested)

This site lies in a shallow basin and forms the grounded, outer forested mat surrounding a small open body of water. The peat surface consists of large hummocks and wet lawns. The site is dominated by a mixture of *Larix laricina* and *Picea mariana* that form an open canopy. Shrubs are abundant, including both *Betula glandulosa* and *B. pumila* var. *glandulifera*. Dwarf shrubs and herbs include an abundance of *Salix pedicellaris*, *Andromeda polifolia*, *Triglochin maritima*, and *Menyanthes trifoliata*. Sedges are not common. The ground layer is composed of a mixture of large populations of *Tomenthypnum nitens* (30%), *Aulacomnium palustre* (30%), *Sphagnum magellanicum* (20%), and *S. warnstorfii* (20%). The site is largely dominated by brown moss, with the larger hummocks dominated by *Sphagnum*. No coring has been done at this or the following sites.

Moderate-rich fen (open)

This site is on a floating mat, surrounding a small open water body, and is adjacent to the previous fen. The peat surface is a uniform carpet that fluctuates in elevation with the water level of the associated lake. The area is dominated by *Carex lasiocarpa*, *C. hysteriana*, and *C. chordorrhiza*, with a ground layer of *Calliergonella cuspidata*, *Drepanocladus vernicosus*, and *Calliergon giganteum*. *Sphagnum* is not present.

Extreme-rich fen (open)

This site is situated in a former north–south drainage channel that presently contains several large bodies of open water, separated by large expanses of rich fens and marsh (Fig. 1). There is usually standing water on the surface of the peatland throughout most of the year. Marl is not present. The site is dominated by *Carex lasiocarpa* and a discontinuous mat of brown moss, with moss plants occurring on the bases of the sedge plants. Species include *Drepanocladus aduncus* and *Aulacomnium palustre*. Vegetatively, the site has some characteristics of a marsh owing to activities of beavers causing seasonal flooding and can be considered transitional between extreme- and moderate-rich fen.

Field methods

Sampling platforms and access boardwalks were installed prior to sampling in the spring of 1989. Surface water was collected from each of the five sites from 10 cm deep pits excavated in the peat. Subsurface waters were collected from PVC pipes by suction after initially purging the pipe of water for each sampling period. PVC pipes were 5 cm in diameter and had a permanent basal cap. Each pipe contained one set of 3.0 mm wide slits cut across the pipe at 0.5-cm intervals along a 10-cm length of pipe. Each site contained three pipes each of which had one set of slits centered at a depth of either 0.5, 1.0, or 1.5 m relative to the peatland surface in the spring of 1989. In between sampling periods the PVC pipes were capped to avoid contamination.

During the ice-free season, four water samples (surface, 0.5, 1.0, and 1.5 m) were collected from each of the five sites at each sampling period. In 1989, 18 samples were taken, while in 1990 water samples were collected 11 times. Water samples for each site and depth were collected in two, 500-mL acid-washed polystyrene bottles and stored at 4°C for elemental and nutrient analyses. Water for pH, conductivity, and alkalinity was collected in 250-mL polypropylene bottles for each site and depth. Water temperatures were measured with a mercury thermometer in the field. Depths to water table in the peat were measured manually with reference to a benchmark, driven to mineral soil in May 1989.

Chemical methods

Water samples for elemental analyses were filtered within 48 h through 0.45-μm HAWP filters and fixed with 0.5 mL of concentrated HNO_3 for a total of 25 mL. Acidified samples were analysed with an inductively coupled argon plasma emission spectrophotometer for base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), total ion species of metals (Al, Fe, Mn, Zn) and elemental forms of sulphur (sulphate and sulfide). Water samples for NH_4^+-N and NO_3^--N were either unfiltered (NH_4^+-N) or filtered using a 0.45-μm HAWP millipore filter (NO_3^--N) and analyzed in triplicate on a Technicon Auto Analyser II within 24 h of field collection.

Total phosphorus, soluble reactive phosphorus, and total dissolved phosphorus were analysed following methods of Bierhuizen and Prepas (1985). Conductivity and pH were determined electronically in the laboratory within 4 h of collection. Conductivity values are standardized to 20°C and corrected for H^+ following Sjörs (1952). Alkalinity was measured with an electrometric titration technique using the phenolphthalein technique (Environment Canada 1979). This measures alkalinity in samples down to a pH of 4.5. Thus, the bog samples from surface waters and those at 0.5 m are designated as zero alkalinity because the pH was always below 4.5.

Statistical methods

Surface water data were compared among peatland, year, and day nested within year, while data derived from the surface and 0.5, 1.0, and 1.5 m depths were compared among peatland, year, day, nested within year and depth. The analyses of variance were performed using the GLM procedure of SAS. The portion of the total variation that could be attributed to each factor was estimated using the VARCOMP procedure (method 1) of SAS (SAS Institute Inc. 1988). The results are reported in percent of the sum of the variance components for all factors.

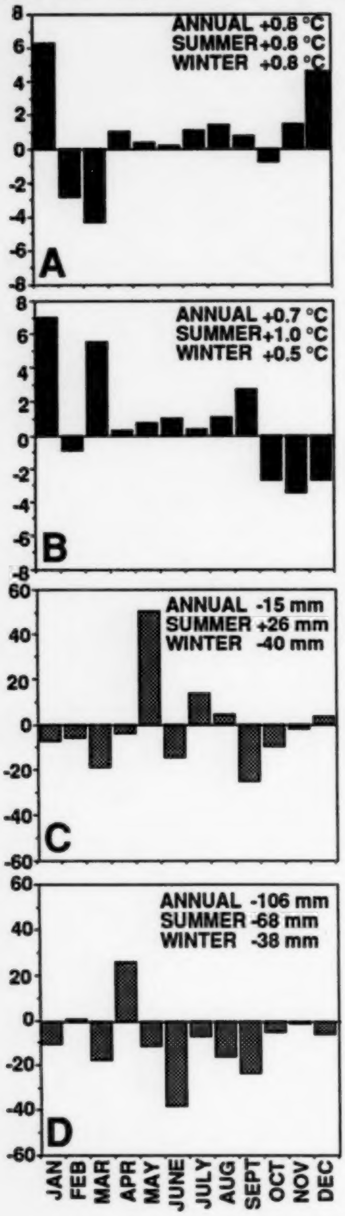
Results

Variation in climate and water levels

During 1989, summer (May–August) air temperatures were somewhat warmer (+0.8°C higher) than the 1951–1980 average, while in 1990 summer air temperatures were 1.0°C higher than the 30-year normals, with the general pattern of variation similar to that in 1989. Six-month (May–October) degree days above 0°C were 2230 in 1989 and 2238 in 1990, compared with the 1951–1980 30-year norm of 2142.

Annual precipitation was slightly less than normal in 1989. However, because of one high precipitation event the overall summer precipitation was somewhat higher

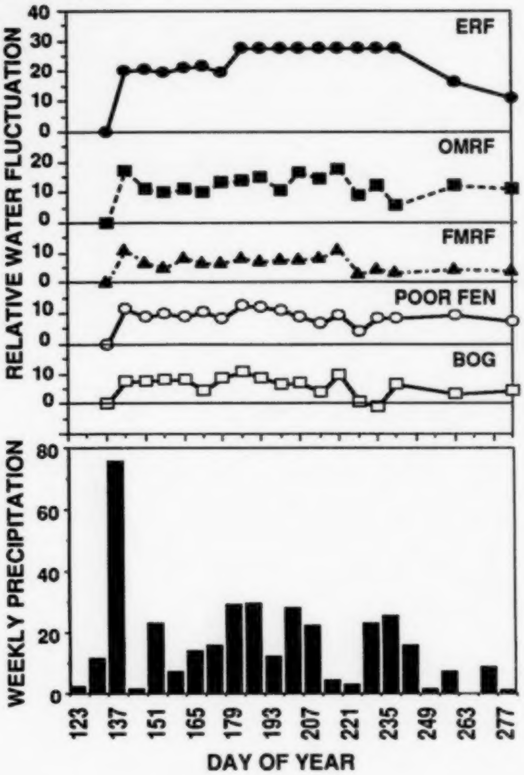
Fig. 2. Monthly mean air temperatures and total precipitation departures from 1951–1980 normals.



than normal. Annual precipitation in 1990 was 106 mm lower than average and the summer amount was 68 mm less than normal (Fig. 2). In summary, 1989 was a relatively normal, although slightly wetter and warmer year, and 1990 was a relatively warm, dry year, especially the first nine months (Fig. 2).

Water levels in the five peatland types generally responded quickly to precipitation events over 60 mm. Weekly precipitation amounts of less than 25 mm elicited little response in water levels. When periods of little or no precipitation lasted longer than 1 week, water levels fell noticeably (Fig. 3).[1] During 1989, the single large precipitation event, days 137–143 (Fig. 3), filled the basins and they remained relatively full for the summer season. In 1990, water levels gradually fell through the growing season in all five peatland types. More variation in water level fluctuation is apparent in the bog compared with the four fen types. The exception is the extreme-rich fen, when the fen was temporarily flooded because of the activities of beaver in 1989.

Fig. 3. Water level fluctuations (cm) over the 1989 sampling period at the five sampling sites compared with weekly precipitation (mm) ERF, extreme-rich fen; OMRF, moderate-rich fen (open); FMRF, moderate-rich fen (forested).



Variation in water temperatures

Overall differences between 1989 and 1990 in surface water temperatures were slight; however, in 1989 maximum surface water temperatures were recorded between days 193 and 235, while in 1990, maximum temperatures were attained earlier in the season (between days 179 and 193). Differences in temperature between peatland type and depth are much more evident than differences between years. Temperatures ranged between 2 and 9°C at 1.5 m depth to as high as 26°C at the surface (Fig. 4). Temperature differences were more evident between the surface and 0.5 m than between any of the other depths. The bog site had consistently lower temperatures at all depths and the insulating ability of the bog is clearly evident when compared to all fen types (Table 2). The acrotelm–catotelm boundary (about 0.5 m) was appreciably colder in the bog than in the fens. Total accumulated degrees for surface waters ranged between 931 and 1195 for the bog in 1989 and 1990 and between 2279 and 2461 for the extreme-rich fen. Rich fens, whether forested or open, had relatively high surface water accumulated degrees (1862–2461), while the *Sphagnum*-dominated poor fen was less (1311–1554) and the bog considerably less (931–1195). The amount of bog surface water was, in general, about 50% less than that in the rich fens. Also differences between years are more evident in the bog than in the fens. Seasonal differences (during the ice-free season) accounts for 48% of the variation in surface water ($p = 0.001$), while wetland type accounts for 32% of the variation (Table 3).

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[1] Because of space limitations only seasonal data for 1989 are shown graphically. Data for both years are available from the senior author. A summary of the chemical data collected for 1989 and 1990 is shown in Table 2.

Variation in water chemistry

pH, alkalinity, and conductivity

Little seasonal variation is evident in pH within sites. This consistent seasonal pattern is strongly contrasted with differences between sites (Table 2) and with depth (Fig. 5). Differences between peatlands account for 96% of the variation in surface water pH (Table 3). The surface water pH of the bog averaged 3.9 in both 1989 and 1990. The pH of the poor fen and open moderate-rich fen were between 5 and 6, while the forested moderate-rich and extreme-rich fens had pH's between 6 and 7 (Table 2). No pattern can be seen between pH and precipitation events. Deeper (1.5 m) in the peat profile, all fen sites had a pH near 6, while the bog is slightly more acidic. The pH in the rich fens increased toward the surface, while in the bog and poor fen the pH decreased toward the surface, showing the increasing ombrotrophy and isolation of the surface peat. The pH of water at the 1.0 and 1.5 m depth was less seasonally variable than the surface waters.

Alkalinity of surface waters of the peatlands ranged between 0 and about 2000 μequiv./L, while at 1.5 m depth it ranged between about 500 and 3500 μequiv./L. There was a consistent increase in alkalinity from bog to poor fen to moderate-rich fen to extreme-rich fen (Fig. 6). Brown moss-dominated rich fens had consistently higher alkalinities than *Sphagnum*-dominated poor fens and bogs. Peatland type accounts for 92% of the variation in alkalinity of the surface waters (Table 3). Differences between years are slight (Table 2). In the poor fen and moderate-rich fens, the surface water alkalinity increased slightly over the season, probably because of evaporative concentration. There was more seasonal variation in surface waters than at depth. As depth increased, differences in alkalinity among sites increased (Fig. 6).

Temporal and spatial patterns of corrected conductivity are quite similar to those of alkalinity, with peatland type accounting for 89% of the surface water variation. In both 1989 and 1990, surface water conductivities increased along the bog-rich fen gradient (Table 2). Conductivities in both surface and subsurface waters were fairly constant over the season (Fig. 7). Differences between years are slight. As with alkalinity, conductivities increased with depth within individual peatlands. For example, the conductivities of the bog increased from about 37 μS/cm

Fig. 4. Differences in water temperature (°C) in the various peatland types during the 1989 sampling period.

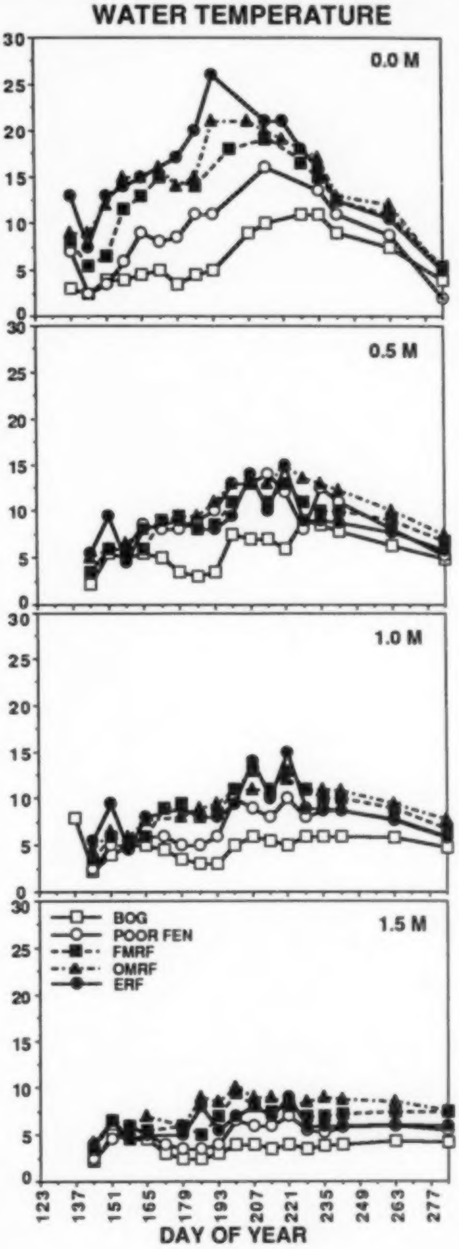


Table 2. Summary of chemical data for surface water for the five peatlands in the 1989 and 1990 sampling periods.

		Bog	Poor fen	FMRF	OMRF	ERF
Water temp. (°C)	1989	6.1 (3.0)	8.4 (4.0)	12.2 (4.5)	14.8 (4.5)	11.9 (5.4)
	1990	7.4 (3.9)	10.0 (4.4)	12.7 (5.1)	14.4 (4.7)	15.5 (7.2)
pH	1989	3.96 (0.07)	5.38 (0.13)	6.28 (0.3)	6.00 (0.25)	6.88 (0.30)
	1990	3.93 (0.29)	5.44 (0.29)	6.21 (0.4)	5.93 (0.31)	6.82 (0.33)
Reduced conduct. (μS/cm)	1989	39.0 (11.0)	48.0 (14.0)	91.0 (19.0)	79.0 (13.0)0)	187.0 (35.0)
	1990	36.0 (14.0)	60.0 (14.0)	82.0 (11.0)	79.0 (8.0)0)	180.0 (37.0)
Ca (μequiv./L)	1989	150.0 (48.0)	294.0 (94.0)	607.0 (175.0)	502.0 (109.0)	1164.0 (326.0)
	1990	215.0 (125.0)	389.0 (124.0)	544.0 (104.0)	489.0 (67.0)	1116.0 (284.0)
Mg (μequiv./L)	1989	59.0 (29.0)	257.0 (86.0)	439.0 (127.0)	392.0 (95.0)	638.0 (144.0)
	1990	115.0 (121.0)	310.0 (72.0)	376.0 (65.0)	353.0 (45.0)	27.0 (133.0)
Na (μequiv./L)	1989	71.0 (15.0)	82.0 (32.0)	111.0 (20.0)	97.0 (15.0)	239.0 (17.0)
	1990	67.0 (18.0)	77.0 (18.0)	73.0 (10.0)	86.0 (26.0)	195.0 (44.0)
K (μequiv./L)	1989	14.0 (10.0)	54.0 (22.0)	34.0 (26.0)	32.0 (16.0)	27.0 (19.0)
	1990	20.0 (16.0)	45.0 (43.0)	25.0 (22.0)	31.0 (29.0)	27.0 (14.0)
Al (μequiv./L)	1989	18.3 (8.5)	10.5 (4.5)	5.6 (3.0)	5.3 (3.1)	6.1 (2.7)
	1990	19.8 (5.8)	9.5 (2.9)	3.7 (1.6)	4.2 (1.5)	2.2 (1.6)
Alkalinity (μequiv./L)	1989	<0 (−)	198.0 (51.0)	764.0 (213.0)	623.0 (154.0)	1716.0 (401.0)
	1990	<0 (−)	371.0 (153.0)	743.0 (120.0)	695.0 (106.0)	1777.0 (315.0)
NO_3 (μequiv./L)	1989	0.41 (0.15)	0.35 (0.12)	0.31 (0.15)	0.33 (0.21)	0.26 (0.13)
	1990	0.82 (0.47)	0.70 (0.39)	0.58 (0.32)	0.51 (0.25)	0.63 (0.51)
NH_4 (μequiv./L)	1989	1.26 (0.60)	0.94 (0.63)	1.04 (0.93)	0.70 (0.52)	0.46 (0.42)
	1990	1.24 (1.37)	1.08(1.15)	1.07 (1.61)	0.68 (0.72)	0.53 (0.87)
SRP (μequiv./L)	1989	0.80 (0.59)	5.10 (2.70)	14.52 (5.53)	0.70 (0.53)	0.71 (0.68)
	1990	0.94 (0.65)	30.58 (30.68)	10.36 (3.98)	7.39 (5.59)	0.69 (0.64)

Note: Data are presented as mean values with the standard deviation in parentheses. OMRF, moderate-rich fen (open); FMRF, moderate-rich fen (forested); ERF, extreme-rich fen.

Table 3. Analysis of variance for the parameters measured in surface waters.

	% total variation					Significance level			
Variable	Peatland	Year	Peatland–year	Day	Error	Peatland	Year	Peatland–year	Day
pH	96.0	−0.2	0.0	2.4	1.9	0.0001	0.6905	0.5350	0.0001
Hydrogon Ion	80.8	0.0	−0.1	0.3	19.0	0.0002	0.3797	0.4425	0.3832
Alkalinity	91.5	0.1	0.1	2.1	6.3	0.0001	0.2918	0.3150	0.0004
Conductivity	88.9	−0.4	0.1	2.5	8.8	0.0001	0.9075	0.1777	0.0017
Temperature	32.2	−1.5	1.7	47.7	19.9	0.0180	0.6628	0.0129	0.0001
Soluble reactive phosphorus	13.5	1.1	30.2	−0.4	55.6	0.3133	0.3448	0.0001	0.5084
Total dissolved phosphorus	6.3	6.4	39.5	0.2	47.5	0.3211	0.2569	0.0001	0.4438
Total phosphorus	10.0	29.9	22.3	7.2	30.6	0.2282	0.0529	0.0001	0.0036
Nitrate	3.1	32.8	1.1	40.0	23.0	0.1690	0.0026	0.1934	0.0001
Ammonium	11.1	−2.3	−4.7	30.4	65.6	0.0005	0.8458	0.9939	0.0001
Calcium	82.3	−0.8	0.5	5.9	12.1	0.0002	0.8936	0.2027	0.0001
Magnesium	79.3	−1.1	1.8	6.6	13.5	0.0009	0.9829	0.0396	0.0001
Sodium	81.5	3.2	3.5	4.4	7.3	0.0018	0.0894	0.0001	0.0001
Potassium	21.4	−3.1	−0.5	36.0	46.2	0.0138	0.7185	0.4844	0.0001
Sulphur	33.6	28.0	9.5	6.0	22.9	0.0504	0.0167	0.0002	0.0027
Aluminum	67.3	−0.5	2.0	14.2	17.0	0.0016	0.3985	0.0521	0.0001
Iron	22.9	−1.8	−1.2	33.1	47.0	0.0081	0.5169	0.5977	0.0001
Manganese	22.1	−1.3	10.7	7.3	61.2	0.1004	0.4562	0.0177	0.0609
Zinc	12.0	6.2	5.8	34.7	41.3	0.1032	0.1455	0.0345	0.0001

Note: Temperature is water temperature; conductivity has been reduced for H^+.

Fig. 5. Differences in pH in the various peatland types during the 1989 sampling period.

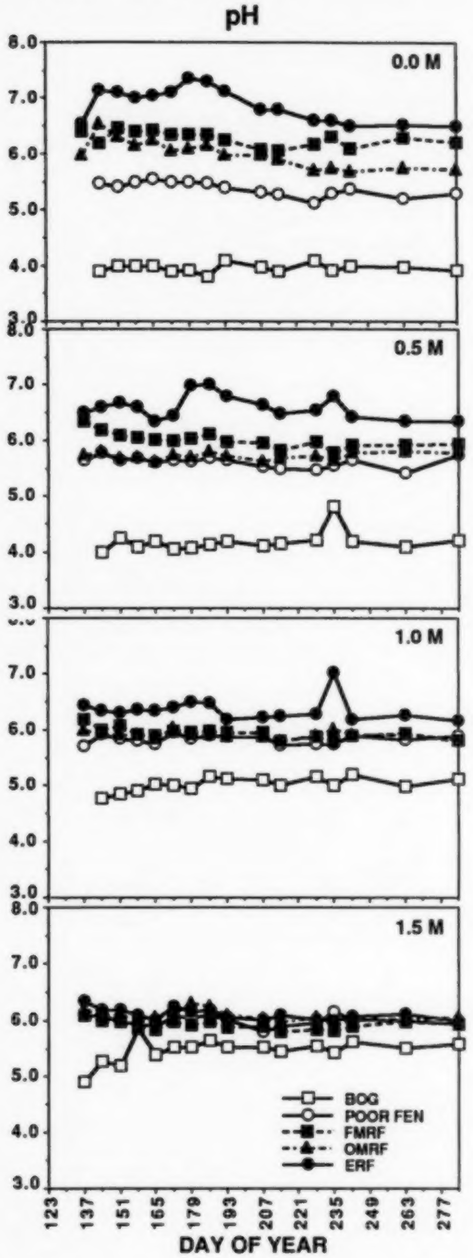
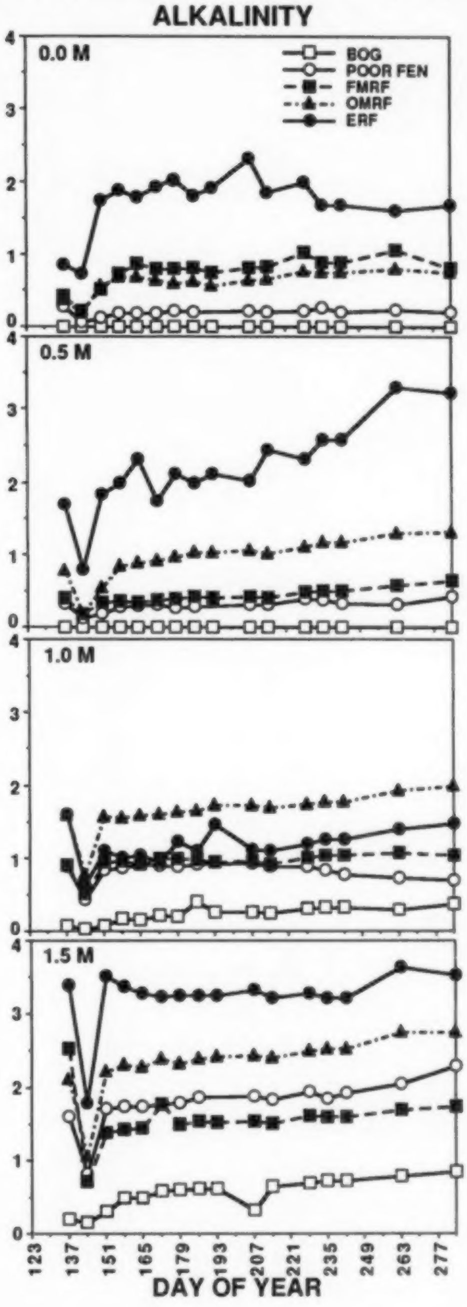


Fig. 6. Differences in alkalinity (mequiv./L) in the various peatland types during the 1989 sampling period.



at the surface to about 108 μS/cm at 1.5 m depth, while those of the extreme-rich fen increased from about 184 to 335 μS/cm at 1.5 m.

Base cation concentrations

Base cation concentrations (sum of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) in surface waters during the ice-free season varied from 355 μequiv./L in the bog to 2017 μequiv./L in the extreme-rich fen. The poor fen and moderate-rich fens were intermediate. Ca/Mg ratios vary from a high of 2.5:1 in the bog to between 1.2–1.8:1 in the fens. Precipitation in the area had a total sum of base cation concentrations of 470

Fig. 7. Differences in corrected conductivity (μS/cm) in the various peatland types during the 1989 sampling period.

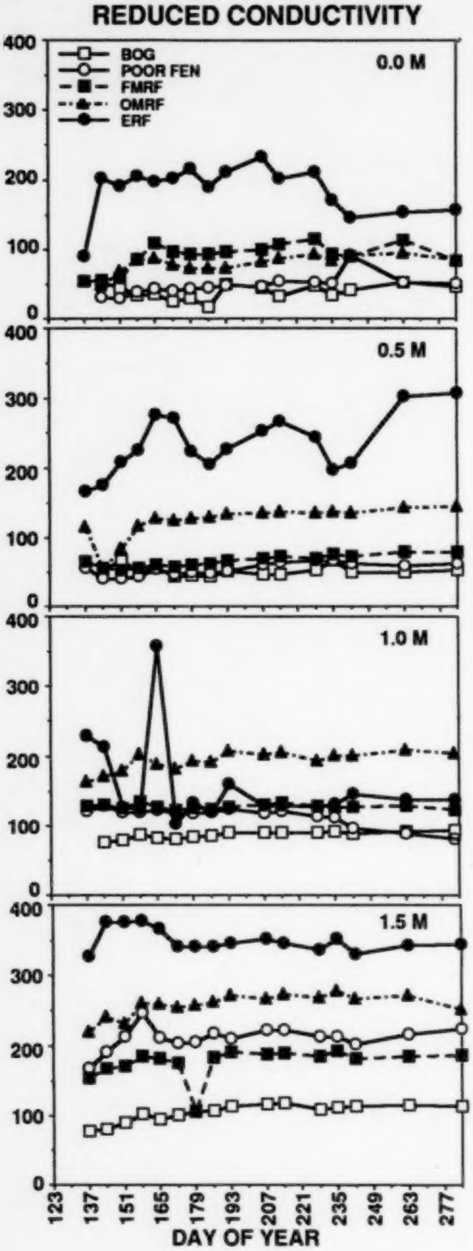
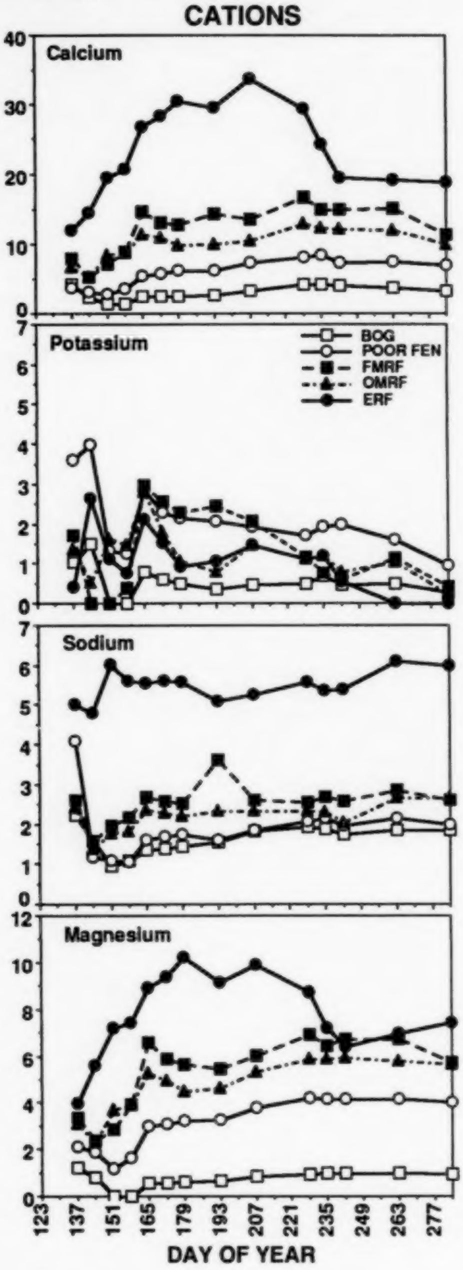


Fig. 8. Differences in base cations (Ca, Mg, Na, K) (mg/L) in the various peatland types during the 1989 sampling period.



μequiv./L (Ca^{2+}, 308 μequiv./L; Mg^{2+}, 77 μequiv./L; Na^{+}, 38 μequiv./L; K^{+}, 47 μequiv./L) and a Ca/Mg ratio of 4:1 (Alberta Environment, unpublished data). These data suggest the following relationships relative to precipitation. Calcium is depleted from bog waters (probably taken up by *Sphagnum*), while it is concentrated in fen waters. Magnesium is also concentrated in fen waters relative to precipitation, but bog concentrations remain similar to those in precipitation. Sodium is always higher in bog and fen waters than in precipitation, while potassium is usually lower in peatland waters than in precipitation. Only the poor fen site had concentrations of potassium in surface water that were as high as precipitation values.

As has been demonstrated by many authors (see Introduction), calcium concentrations increase along the bog-rich fen gradient (Table 2). The magnesium and sodium concentrations exhibit the same pattern. Potassium concentrations do not show this gradient. Surface water potassium concentrations were highest in the poor fen and lowest in the bog, while the three rich fen sites were intermediate. Whereas calcium removal through cation exchange may account for the lower values in the bog, calcium enrichment from ground water may account for the increase in calcium along the poor to rich fen gradient. Local and regional studies of groundwater in the area have documented the calcareous nature of the groundwater (Borneuf 1972). At all sites, calcium was the dominant ion, followed by magnesium, sodium, and potassium (Table 2). Peatland type accounts for 79–82% of the surface water variation for calcium, magnesium, and sodium, but only 21% of the variation for potassium (Table 3).

On an equivalent basis, the bog contained about 16% of the calcium of the extreme-rich fen, while the poor fen contained about 30%. Magnesium differences along the bog–fen gradient were similar (the bog with 14% and the poor fen with 45% of the extreme-rich fen). However, sodium concentrations for the bog were relatively higher (32% of those of the extreme-rich fen). Trends in the distribution of potassium were different, with the poor fen having the highest overall concentrations and the bog the lowest concentrations of this ion. If the four fen sites are averaged, then the bog surface waters contain 39% of the potassium contained in the fen waters. Thus the bog contains proportionally less calcium and magnesium, and more sodium and potassium.

There were large fluctuations in concentrations of calcium, magnesium, and sodium during the growing season (Fig. 8). At the extreme-rich fen and moderate-rich fen sites, there was a general increase in surface water concentrations during the growing season, while at some sites in some years there was an initial spring peak followed by a decline in concentration. This may be related to evaporative concentration during the drier mid-summer period. In the bog, the seasonal patterns of calcium, magnesium, and sodium generally showed a high spring concentration, a sharp decline in early summer, followed by a general rise over the summer. Potassium concentrations followed this same pattern at all sites, suggesting inputs from the spring snowmelt followed by vegetative uptake during the summer. Seasonal variation accounts for 36% of the variation in potassium concentration, while wetland type accounts for 21% of the variation (Table 3).

Metal concentrations

Seasonal profiles for zinc, manganese, iron, and aluminum were much more variable than those for the cations (Fig. 9). Zinc concentrations were not significantly different between peatland sites; however, there appears to be a relationship between high concentrations and lack of precipitation (Figs. 2, 3, and 9). Season accounts for 35% of the variation in surface water zinc concentration (Table 3). Manganese concentrations were variable in the fen profiles, while it was generally below detection levels in bog surface waters. Mean annual concentrations of manganese were the lowest in the bog, higher in the poor fen and extreme-rich fen, and highest in the forested moderate-rich and open

Fig. 9. Differences in metals (Al, Fe, Mn, Zn) (mg/L) in the various peatland types during the 1989 sampling period.

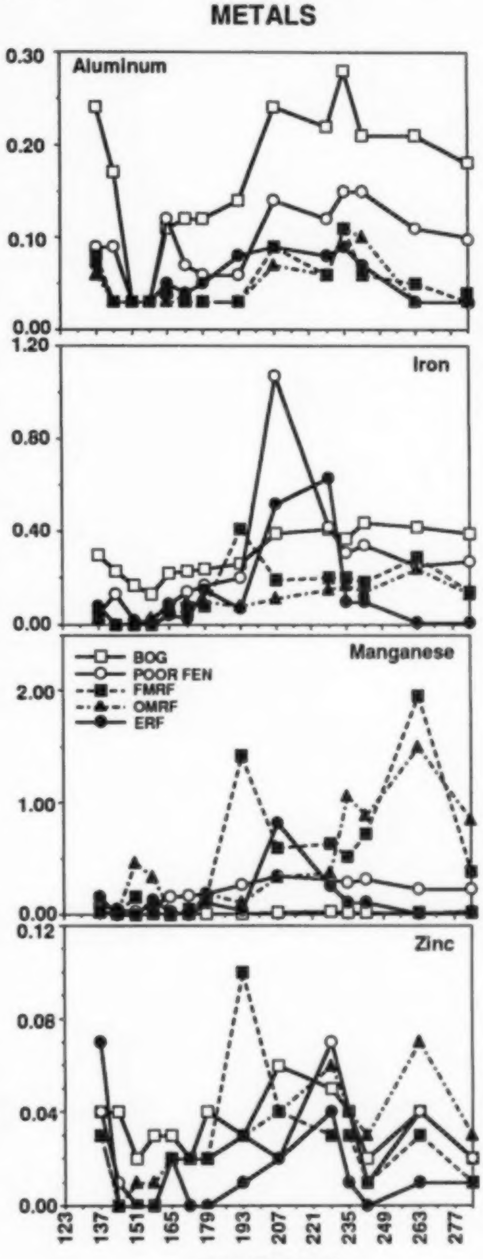
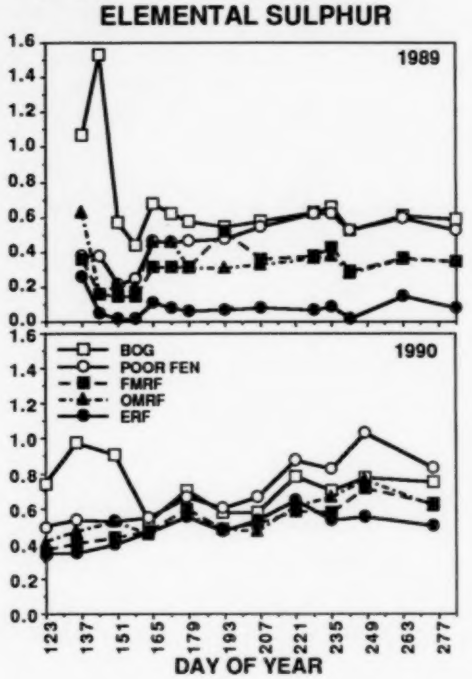


Table 4. Analysis of variance for the various parameters measured in surface water and pore water at 0.5, 1.0, and 1.5 m depth.

Variable	% total variation										
	Peatland	Year	Peatland–year	Depth	Peatland–depth	Year–depth	Peatland–year–depth	Day	Peatland–day	Depth–day	Error
pH	69.6	0.0	−0.1	−2.3	26.7	0.2	0.1	2.5	0.7	0.5	2.0
Hydrogen ion	30.2	0.0	2.5	−0.4	45.1	0.0	1.7	0.3	7.8	0.0	13.0
Alkalinity	50.1	0.1	−0.3	31.1	12.8	−0.1	0.9	2.0	0.6	0.5	2.4
Reduced conductivity	41.4	−0.1	−0.1	38.3	14.2	0.3	0.6	0.3	0.5	0.4	4.3
Water temperature	17.2	−1.1	−3.7	12.1	1.7	2.5	24.8	21.7	1.7	8.6	14.6
Soluble reactive phosphorus	23.7	2.5	−1.4	15.3	35.9	1.1	6.5	−0.7	1.4	0.6	15.1
Total dissolved phosphorus	27.0	1.0	−1.7	15.6	38.0	1.6	6.7	−0.5	1.7	0.3	10.4
Total phosphorus	23.3	−2.8	−1.6	6.4	37.8	10.7	6.0	1.3	1.8	3.0	14.2
Nitrate	0.6	39.3	−0.5	0.6	0.4	0.0	1.8	37.6	2.1	6.7	11.3
Ammonium	25.4	0.1	0.1	20.6	49.4	0.0	0.5	0.3	0.4	0.4	2.8

Fig. 10. Differences in elemental sulphur (mg/L) in the various peatland types during the 1989 and 1990 sampling period.



moderate-rich fens. In contrast, both iron and aluminum were more abundant in bog surface waters. Despite the bog–fen gradient, peatland type only accounts for 23% of the surface water iron variation, while season accounts for 30% of the variation (Table 3). Aluminum concentrations followed patterns similar to those for iron, with highest mean annual concentrations in the bog and poor fen. Moderate-rich fens and the extreme-rich fen concentrations of aluminum were nearly identical. Peatland type accounts for 67% of the variation in surface water aluminum concentration (Table 3).

Elemental sulphur concentrations

Elemental sulphur was highest in the bog and poor fen and lowest in the extreme-rich fen, with the other fens being intermediate (Fig. 10, Table 2). Peatland type accounts for 34% of the variation in surface water concentration, although analysis of variance indicates that this is not significant (Table 3). Differences between sites were more obvious in 1989, which was the wetter of the 2 years. Sulphur concentrations for the fen sites in 1989 were lower than those in 1990 (the drier of the 2 years). Bog values were more constant, but still higher in 1990. Differences between years account for 28% of the variation in surface water sulphur concentration (Table 3).

Nutrient concentrations

Nitrate (as NO_3^--N) concentrations were usually low, generally less than 15 μg/L (Table 2). No significant differences

Fig. 11. Differences in nitrate (μg/L) in the various peatland types during the 1989 sampling period.

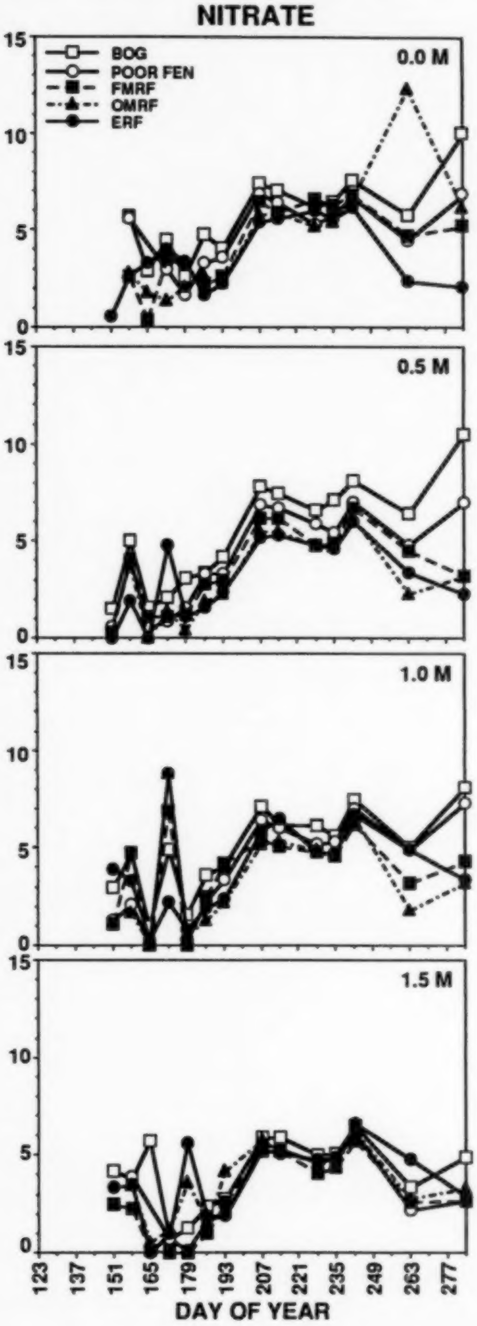
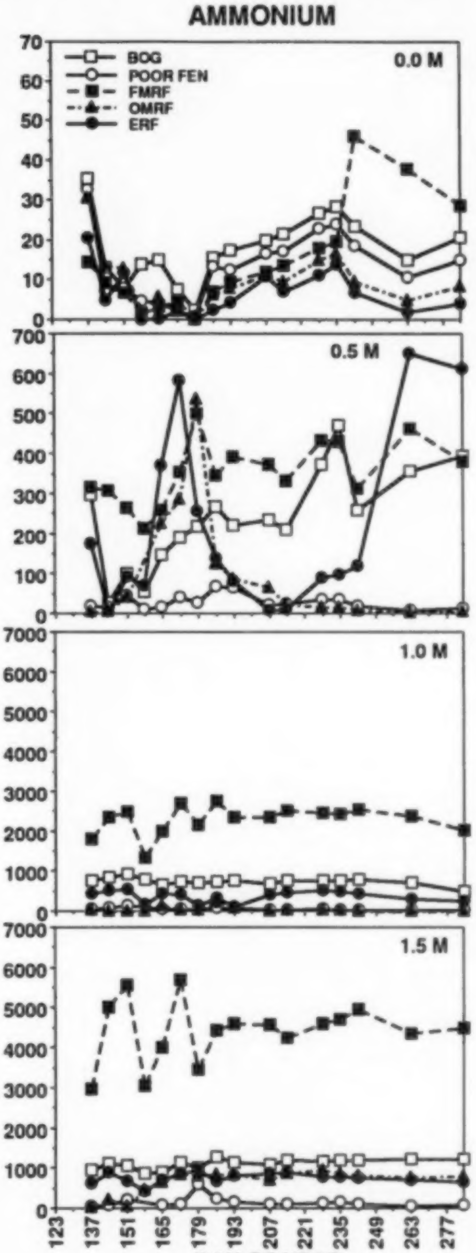


Fig. 12. Differences in ammonium (μg/L) in the various peatland types during the 1989 sampling period.



are apparent among the five sites either in surface water or in pore water (Tables 3 and 4), although the bog waters sometimes contained slightly more nitrate. Likewise, no differences exist with depth at the five sites (Table 4). Peatland type only accounts for 3% of the variation in surface water concentration. Seasonal differences account for 40% of the variation and year accounts for 33% of the variation in surface water (Table 3). There was considerable seasonal variation at all sites (Fig. 11). Nitrate concentrations fluctuated together in all peatlands and at all depths

Fig. 13. Differences in soluble reactive phosphorus (μg/L) in the various peatland types during the 1989 sampling period.

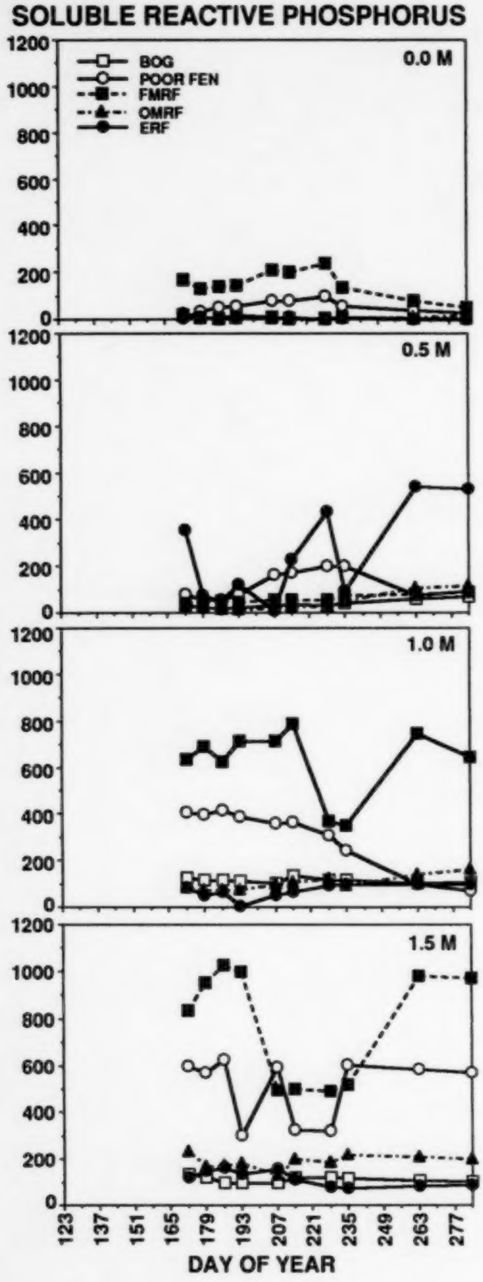
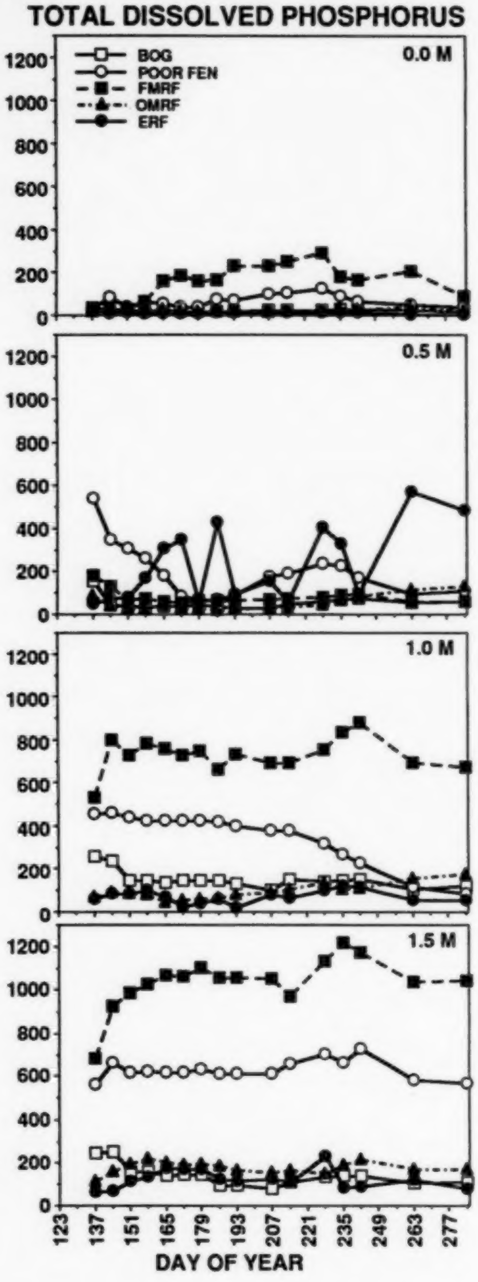


Fig. 14. Differences in total dissolved phosphorus (μg/L) in the various peatland types during the 1989 sampling period.



(Fig. 11). Seasonal variation was slightly more pronounced at the surface and decreased with depth. There was some indication that seasonal variation could be associated with precipitation (Figs. 2, 3, and 11) and that there were higher NO_3^--N concentrations during 1990, the year with lower precipitation (Table 2).

Ammonium (as NH_4^+-N) levels of the surface waters were generally low, with the mean annual concentrations highest in the bog and decreasing through the fen series to their lowest values in the extreme-rich fen (Table 2). Variation because of peatland type accounts for only 11% of the differences, while seasonal variation accounts for 31% of the variation in the surface waters (Table 3). Ammonium concentrations were variable at the surface, with a general pattern consisting of a spring peak, low summer values, and a gradual increase toward fall (Fig. 12). Surface water concentrations of ammonium were generally higher than concentrations of nitrate in all peatlands, with ratios of NH_4^+-N/NO_3^--N ranging from 1.3–3.3:1. Only in the extreme-rich fen were the concentrations of NH_4^+ and NO_3^- similar. Whereas NO_3^- concentrations changed little with depth, ammonium concentrations were generally much higher at depth than they were in surface waters (Table 2). The forested moderate-rich fen had the highest values of ammonium at every depth. The bog and extreme-rich fen had intermediate values, while the open moderate-rich fen and the poor fen had the lowest values. In general, NH_4^+ concentrations at 1.0 and 1.5 m depths varied little over the season and between years.

Soluble reactive phosphorus (SRP) in surface waters was usually less than 150 μg/L and highest in the poor fen and forested moderate-rich fen (Table 2). It was lowest in the bog and extreme-rich fen. Phosphorus concentrations generally increased with depth in all peatland types (Fig. 13). In some sites there were occasional seasonal fluctuations, but in general seasonal variation was small (Fig. 13).

In general, patterns of variation and absolute amounts of total dissolved phosphorus (TDP) and total phosphorus (TP) are similar to those of SRP (Figs. 14 and 15). Total phosphorus concentrations in surface waters were highest in the forested fen, poor fen, and open moderate-rich fen and lowest in the bog and extreme-rich fen (Table 2). Peatland type accounts for only 10% of the variation in TP concentration, while year accounts for 30% and peatland–year interaction accounts for 23% of the variation in surface waters (Table 3). Concentrations deeper in the peat profile were generally higher than in surface waters, but in 1990 the subsurface waters were sometimes lower in concentration.

N/P ratios (calculated as $NH_4^+ + NO_3^-$/SRP) suggest that nitrogen is very limiting in peatland waters, with N/P ratios varying from 4:1 in bog and extreme-rich fen surface waters to 0.2:1 in forested moderate-rich fen waters. The variation in the data is largely due to variation in P not N. Variation in nitrogen components in surface waters is mostly accounted for by seasonal differences (40%, 30%), whereas variation among phosphorus components is accounted for mainly through peatland–year interactions (30%, SRP; 40%, TDP; 22%, TP) (Table 3).

Relationships between surface water components

Corrected conductivity in these continental peatlands is strongly correlated ($p = 0.0001$) with concentrations of calcium. This is confirmed by strong correlations between alkalinity and corrected conductivity ($p = 0.0001$). Likewise, there is a strong correlation ($p = 0.0001$) between the log of the pH and corrected conductivity. The peatland types are clearly separated along these correlated chemical gradients, with amounts increasing along the bog-rich fen gradient (Fig. 16). These correlations remain constant between years. It is clear from Fig. 16 that the five peatland types studied form four nodes along the pH-corrected conductivity regression line. These nodes on this chemical gradient correspond well to the vegetation-based division of our five sites.

Fig. 15. Differences in total phosphorus (μg/L) in the various peatland types during the 1989 sampling period.

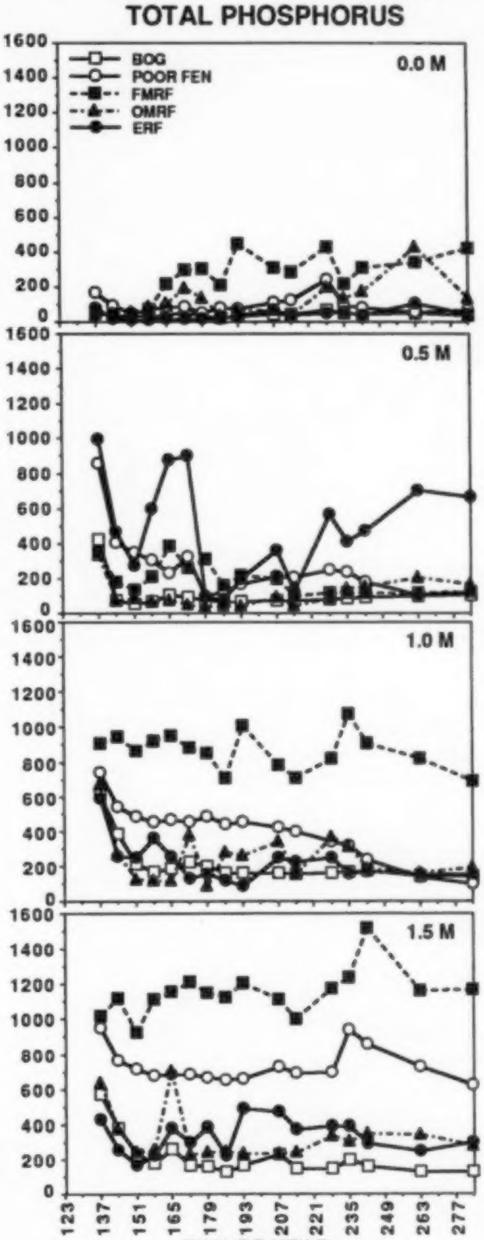


Fig. 16. Relationship between corrected conductivity (µS/cm) and calcium, alkalinity, and pH for surface waters along the bog – rich fen gradient.

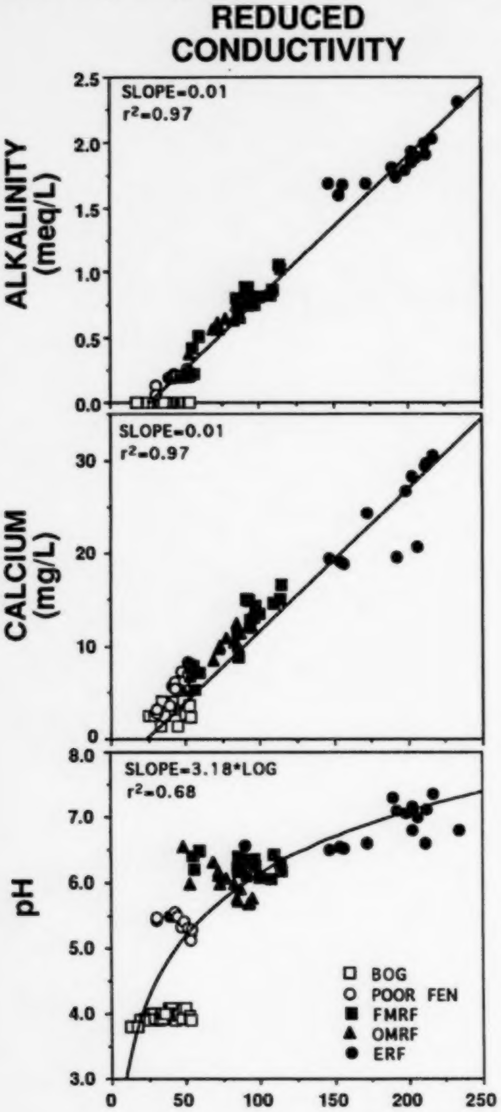
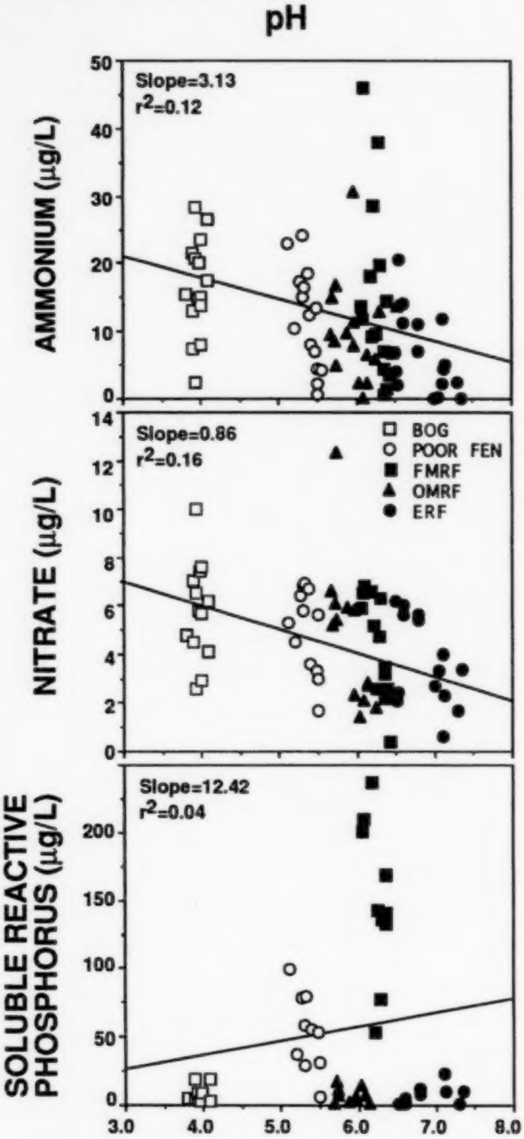


Fig. 17. Relationship between pH and ammonium, nitrate, and soluble reactive phosphorus for surface waters along the bog – rich fen gradient.



Concentrations of ammonium and nitrate are either not or less significantly correlated to the bog-rich fen gradient ($p = 0.169–0.0005$). The slopes are negative, with higher concentrations in the bog than in the rich fens (Fig. 17). Seasonal variation is high. Whereas in the wetter year (1989) ammonium concentration was high and nitrate low, in the drier year (1990) the reverse was true (Table 2). Likewise, SRP is not significantly correlated with the bog-rich fen gradient ($p = 0.313$). Careful inspection of the data for SRP, TDP, and TP suggests that the highest concentration of phosphorus is found between pH 5.2–6.2.

Concentrations of sulphur, aluminum, and iron are all significantly correlated with pH ($p = 0.0001$). Sulphur shows distinct differences between years in the fens, but not in the bog (as indicated by the significant peatland–year interaction, $p = 0.002$ (Table 3)). Thus during the dry year (1990) concentrations of sulphur were considerably higher in the fens than in the relatively wet year (1989). Aluminum and iron show very similar distributions relative to pH. Both are more abundant as pH decreases, show few differences between the 2 years, and have considerable annual variation (Fig. 18).

Relationships of surface water chemistry to pH show three patterns: (*i*) a general positive and highly significant correlation with little seasonal variation within peatland types (base cations, alkalinity, and corrected conductivity), (*ii*) less significant correlation with strong seasonal variation within peatland types (nutrients, N and P) , and (*iii*) a general negative and highly significant correlation with some seasonal variation in peatland types (metals, S).

When depth (at 0.5, 1.0, and 1.5 m) is considered along with peatland type, and season (Table 4), it is clear that pH, H^+, alkalinity and corrected conductivity are still significantly related to peatland type, while NH_4^+-N and all components of P are affected by interactions of peatland type and depth. Nitrate is the only component affected by yearly variation (sulphur was not sampled at depth).

Discussion and conclusions

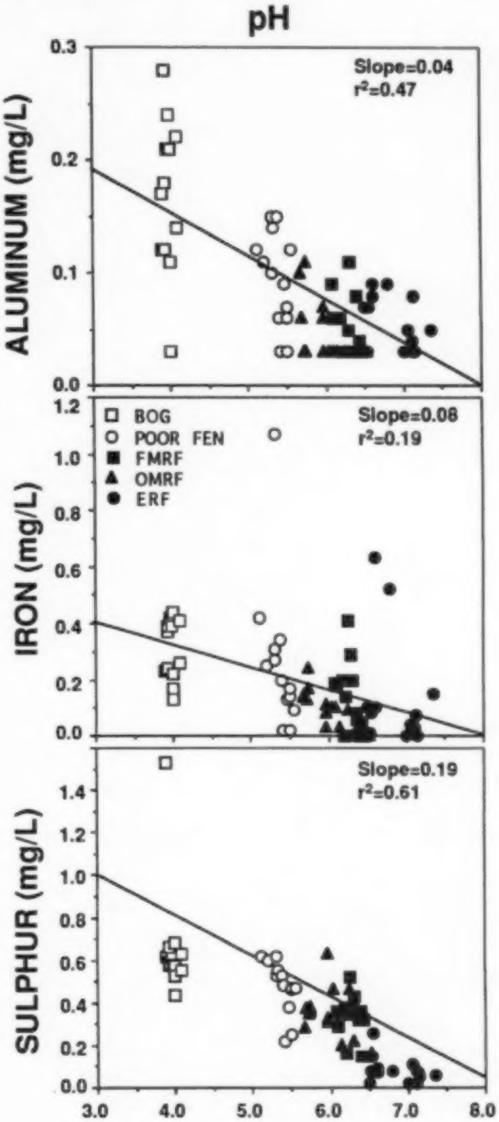
The chemical parameters described above can be used to answer several fundamental questions about the biogeochemistry of northern peatland ecosystems. The present data set is particularly useful because the five sites studied represent the entire spectrum of peatland types along the bog-rich fen gradient and are located in close proximity to one another under similar climatic and topographic regimes. We will address the four questions posed in the objectives.

What components of water chemistry can be used to corroborate the vegetation-based classification into bog and fen types?

Three chemical parameters (pH, alkalinity, and calcium) form the primary chemical gradients associated with the bog-rich fen gradient (Gignac et al. 1991). Gorham et al. (1987) have also shown the relationships of pH to calcium and alkalinity that are present in peatland surface waters. Also important along this gradient are magnesium, and to a lesser extent, sodium. In continental peatlands (including our sites), sodium increases along the bog-rich fen gradient and often closely parallels calcium (Malmer 1962, Gorham et al. 1985). By contrast, in maritime regions sodium is as high or higher in bogs than in fens (Vitt et al. 1990).

The ionic gradient of peatlands is largely controlled by water source, with bogs receiving water solely from precipitation and fens receiving water from precipitation as well as runoff and (or) subsurface interflow and groundwater flow in differing proportions. Ions are generally concentrated relative to the source because of evaporation, except in *Sphagnum*-dominated systems where preferential cation exchange occurs resulting in a loss of certain cations (e.g., Ca^{2+}). As base cation concentrations are dependent on water source, peatlands along the bog-rich fen gradient will have different base cation concentrations depending on their location (e.g., oceanic vs. continental systems, siliceous vs. carbonate-dominated systems). Thus, although base cations have long been used as a defining feature of peatland types, it is apparent from these data that the chemical characters that are of critical importance are acidity (pH) and alkalinity (HCO_3^-). These two parameters are more useful in characterizing peatlands than base cations.

Fig. 18. Relationship between pH and elemental sulphur, aluminum, and iron for surface waters along the bog – rich fen gradient.



Unlike pH, alkalinity, and the three base cations (Ca^{2+}, Mg^{2+}, and Na^+), the variations in potassium, nitrogen, and phosphorus concentrations are more related to season, year or peatland–year interactions than the effect of peatland. Nutrient conditions of peatlands are not related to water source as in the case of pH, alkalinity, corrected conductivity, and the base cations Ca^{2+}, Mg^{2+}, and Na^+. The seasonal, yearly or peatland–year interactions suggest that factors regulated by time are controlling nutrient levels. Correlations between nitrate and metals with precipitation suggest that available moisture/redox potential coupled with decomposition and mineralization are the most important factors responsible for nutrient levels in peatlands.

Concentrations of N, P, and K in surface waters cannot be used to categorize nutrient conditions along a bog–fen gradient in peatlands.

Is there significant temporal variation in water chemistry?

Surprisingly, there are few data published on seasonal or annual variations in water chemistry of peatlands, especially along the bog–fen gradient. Vitt (1990) showed variation in pH over growing seasons for a bog, poor fen, and several rich fens, and Bayley et al. (1987) showed weekly concentrations for pH, NO_3^-, and SO_4^{2-} for a basin bog and associated fen lag from northwestern Ontario. Vitt and Bayley (1984) showed distinct differences in water chemistry in several bogs and poor fens in NW Ontario. Urban and Eisenreich (1988) showed seasonal concentrations of nitrogen in Marcel Bog in Minnesota. Farther south in West Virginia, Wieder (1985) presented seasonal patterns for 11 parameters (measured monthly between April and December, 1980) from the surface and from 30 cm depth. His data show considerably more variation than ours; however, his sites are at the southern boundary of bog–fen systems and were subjected to late summer drought and generally snow-free early winter conditions. During the growing season, his values are more uniform and similar to ours.

Most chemical parameters show some seasonal fluctuation in surface waters. However, our data suggest relatively constant concentrations for hydrogen ion and ammonium. Alkalinity and corrected conductivity are relatively constant throughout the season except in the extreme-rich fen. Sulphur is relatively constant except in the bog where a spring pulse and summer drought may have increased concentrations. Soluble reactive phosphorus is relatively stable throughout the season except in the poor fen. The base cations (Ca^{2+}, Mg^{2+}, and K^+), metals (Al, Fe, Mn, Zn), and total phosphorus showed a wide range in concentrations throughout the season in most peatland types in both 1989 and 1990.

Comparisons between 1989 and 1990 suggest greater variation in water level fluctuation in 1990 (the drier year) and lower water levels. During periods with a low water table, a relatively larger proportion of the upper peat column would be aerobic for longer time periods. This variability in 1990 compared with 1989 was observed in several chemical components, especially sulphur, nitrate, and phosphorus (SRP and TP). The drought in late summer 1990 also resulted in pHs lowered by 0.4–0.5 units throughout the water column. High precipitation events in both years increased pH to a lesser degree. These effects are not reflected in the seasonal profiles of corrected conductivity, alkalinity, or base cations. Nitrate variation reflects in part precipitation patterns, with higher precipitation events resulting in increases in nitrate and periods of drought reducing concentrations.

Seasonal variation is least in bogs and greatest in rich fens along the acidity–alkalinity gradient. However, in no cases did pH vary more than one pH unit over the course of the season. Seasonal variability in our extreme-rich fen site may be due to activities of beavers and subsequent dam removal, indicating the strong influence that flooding events can have on water chemistry.

These data suggest that when sampling peatland surface waters, a single sampling period will not be representative of the peatland for all the chemical components. For those components that are relatively constant throughout the season (pH, alkalinity, corrected conductivity, ammonium, sulphur, and soluble reactive phosphorus) a one-time sampling period will adequately represent the peatland surface waters. When the base cations (Ca^{2+}, Mg^{2+}, K^+), metals (Al, Fe, Mn, Zn), and total phosphorus are considered, a one-time sampling period will not adequately represent the surface water of the peatland. Sampling of peatland surface waters will also not be representative during times of drought or flooding.

Does subsurface water chemistry differ from that of the surface water?

Subsurface water chemistry at all sites generally reflects the influence of mineral-rich ground water inflow through the peatland basin. Alkalinity and corrected conductivity increase with depth at all sites. The pH of the moderate-rich fens and the extreme-rich fen remains about the same with depth, while the pH of the bog and poor fen increases with depth indicating that at depths greater than 1 m the peat profile is affected by minerotrophic waters.

Whereas nitrate concentrations remain constant throughout the profile, ammonium strongly increases with depth (except in the poor fen). At 0.5 m depth, three of the five sites (including the bog) had concentrations of ammonium of greater than 300 μg/L. Phosphorus concentrations were also generally higher in the drier layers. Whether or not these relatively large pools of ammonium and phosphorus are utilized by plants is unknown. Backeus (1990) reported more than 50% of the fine root mass in the upper 10 cm in a wet year, while in a dry year he suggested that the root mass may penetrate to greater than 40 cm, and therefore the roots may utilize this large pool of ammonium and phosphorus.

More striking than the differences in depth profiles of nutrients are the temperature profiles. Water temperatures at all sites decreased down through the peat column, reflecting the insulating effects of peat. Peat in bogs is known to promote low soil temperatures resulting in the development of permafrost in the boreal forest. In fact, vascular plant production in the bog may be in part limited not only by nutrients but also by low water temperatures within the root zone.

Are spatial and temporal patterns of nutrients different from those of the base cations, metals, and elements?

Alkalinity, corrected conductivity, pH, Ca^{2+}, Mg^{2+}, and Na^+ increase along the bog – rich fen gradient. They are generally highly correlated with one another and do not appreciably vary either over the growing season or between seasons. Thus, the most significant source of variation for these chemical components is peatland type.

Metals decrease along the bog – rich fen gradient, as has been observed elsewhere (Helmer et al. 1990). They are highly variable within peatland types over the season, but similar between years. There is no evidence in these data that indicates relationships with yearly variation in water level fluctuations. Both Al and Fe concentrations are enriched in bogs and this enrichment is a function of the high DOC and organic–acid–metal binding that occurs at low pH (Helmer et al. 1990).

Elemental sulphur also decreases along the bog–fen gradient, but it is much less variable over the growing season than metals. Yearly differences are significant, particularly for extreme-rich fen sites. The most significant sources of variation are year and peatland type.

In general, nutrients show either no relationship or a slight decrease along the bog-rich fen gradient. Among the nutrients, ammonium and phosphorus increase in concentration and concomitantly show less seasonal variation with depth. Surface concentrations of ammonium are somewhat higher in the bog, while those of phosphorus are higher in the middle pH range. Nitrate occurs in very low concentrations at all depths and shows considerable seasonal variation at all depths. The most significant source of variation for nutrients is peat profile depth.

Acknowledgments

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Effects of water chemistry and temperature on radiocesium dynamics in rainbow trout, *Oncorhynchus mykiss*

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Abstract: We determined the effects of temperature (8 and 16°C) and water concentrations of potassium (0.01–3.4 mmol/L), sodium (0.4–3.0 mmol/L), and calcium (0.3–2.9 mmol/L) on the dynamics of ^{137}Cs in rainbow trout (*Oncorhynchus mykiss*). Trout were fed food labelled with ^{137}Cs, and ^{137}Cs retention was monitored by whole body gamma counting (70 d). Assimilation of ^{137}Cs did not vary significantly over the range of concentrations of K^+, Na^+, and Ca^{2+} employed, with an average value of 65 ± 3% (mean±SE). The elimination of ^{137}Cs was significantly affected by K^+, with the elimination rate constant of trout held at the highest K^+ concentration ($0.021 \pm 0.001\ d^{-1}$ at K^+ of 3.4 mmol/L) roughly double that observed in trout at lower K^+ ($0.011 \pm 0.001\ d^{-1}$ at $K^+ \leq 0.3$ mmol/L). Elimination of ^{137}Cs was also significantly affected by temperature, with the increase in elimination rate over the range of 8 and 16°C corresponding to a Q_{10} of ≈1.7. Although the effect of K^+ on accumulation of ^{137}Cs in trout was statistically significant, this effect, by itself, can explain only a small fraction of the broad range of ^{137}Cs concentration ratios reported for wild fish.

Résumé : Nous avons mesuré les effets de la température (8 et 16°C) et des concentrations dans l'eau du potassium (0,01–3,4 mmol/L), du sodium (0,4–3,0 mmol/L) et du calcium (0,3–2,9 mmol/L) sur la dynamique du ^{137}Cs chez la truite arc-en-ciel (*Oncorhynchus mykiss*). Nous avons nourri les truites avec des aliments marqués au ^{137}Cs et mesuré la rétention de cet élément par comptage des radiations gamma sur l'ensemble du corps (70 d). L'assimilation du ^{137}Cs ne variait pas de façon significative dans la plage des concentrations de K^+, Na^+ et Ca^{2+} employées, la valeur moyenne se situant à 65 ± 3% (moyenne ± ET). L'élimination du ^{137}Cs était affectée de façon significative par K^+, la constante du taux d'élimination chez les truites maintenues à la plus forte concentration de K^+ ($0,021 \pm 0,001\ d^{-1}$, K^+ de 3,4 mmol/L) étant grossièrement le double de celle qui a été observée chez des truites à des concentrations plus faibles de K^+ ($0,011 \pm 0,001\ d^{-1}$, $K^+ \leq 0,3$ mmol/L). L'élimination du ^{137}Cs était aussi affectée de façon significative par la température, l'accroissement du taux d'élimination sur la plage de 8–16°C correspondant à un Q_{10} de ≈1,7. Bien que l'effet de K^+ sur l'accumulation du ^{137}Cs chez les truites ait été statistiquement significatif, cet effet, en lui-même, ne peut expliquer qu'une petite fraction de la large plage de concentrations de ^{137}Cs signalées pour les poissons sauvages.

[Traduit par la Rédaction]

Introduction

Cesium-137 (^{137}Cs) is of particular concern among the radionuclides released to the aquatic environment by nuclear operations and weapons testing, because of its high yield in uranium-235 fission, its relatively long half-life (30 years), and its tendency to accumulate in aquatic biota (Vanderplong et al. 1975; Butler 1980). Consumption of contaminated fish is one of the principal paths through which man may be exposed to environmental ^{137}Cs. In 1987, 1 year after the accident at Chernobyl, the ^{137}Cs concentrations in fish (100 g perch) from approximately 14 000 lakes in Sweden exceeded the Swedish guideline of 1500 Bq/kg, and restrictions were placed on the consumption of fish from these lakes (Håkanson et al. 1992).

Accumulation of ^{137}Cs in freshwater fish varies substantially among waterbodies, with reported concentration ratios (the steady state ratio of ^{137}Cs concentrations in fish and water) ranging from less than 100 to greater than 10 000 (Vanderplong et al. 1975; Poston and Klopfer 1988). A wide range of studies has shown that the ionic composition of the water is a key factor affecting ^{137}Cs accumulation with the greatest accumulation observed in waters with low concentrations of both potassium (K^+) and those ions which collectively contribute to conductivity and total hardness (Bryan 1963; Davis 1963; Kevern and Spigarelli

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1971; Vanderplong et al. 1975; Poston and Klopfer 1988; Håkanson et al. 1992).

Our understanding of the mechanisms through which the chemistry of the water could affect ^{137}Cs accumulation in fish is confounded by the fact that diet, rather than water, appears to be the dominant source of ^{137}Cs to freshwater fish in "naturally contaminated" environments (Kolehmainen 1972; Vanderplong et al. 1975; Thomann 1981). Potassium and other ions could affect ^{137}Cs accumulation within aquatic ecosystems either through specific effects on the uptake or elimination of ^{137}Cs in aquatic biota, or through general effects on the distribution and partitioning of ^{137}Cs. Effects on the uptake or elimination of ^{137}Cs in aquatic biota at lower trophic levels could then be amplified by passage through the food chain (Thomann 1981).

The effects of K^+ on the accumulation of ^{137}Cs are thought to derive from their chemical similarity, as both cesium (Cs^+) and potassium are alkali metals. In natural waters, where concentrations of K^+ are several orders of magnitude greater than Cs^+, K^+ is thought to serve as a "non-isotopic carrier" for ^{137}Cs (Davis 1963; Vanderplong et al. 1975). If K^+ serves as a non-isotopic carrier for ^{137}Cs, high concentrations of K^+ would effectively dilute the ^{137}Cs pool, and reduce net accumulation of ^{137}Cs, either by decreasing uptake or increasing elimination of ^{137}Cs. In fish, assimilation of ^{137}Cs from the diet has been observed to vary significantly with diet composition, especially with clay content (Kolehmainen 1972; Forseth et al. 1992), but the effects of water chemistry on the accumulation of dietary ^{137}Cs have not been investigated. Detailed studies of the effects of K^+ on ^{137}Cs elimination in aquatic organisms are also limited, although Bryan (1963) observed a reduction in the rates of loss of both ^{42}K and ^{137}Cs from the brackish water invertebrates, *Potamopygus* and *Sphaeroma*, when the animals were exposed to media with lower K^+ concentrations. Both Sanders and Kirschner (1983) and Eddy (1985) reported a reduction in the branchial efflux of K^+ from rainbow trout at lower concentrations of K^+. If K^+ serves essentially as a non-isotopic carrier for ^{137}Cs, a parallel decrease in ^{137}Cs elimination would be expected at lower K^+ concentrations. Other water quality parameters, particularly Ca^{2+}, could affect ^{137}Cs accumulation in fish through their influence on the permeability of the gills to molecules and ions (Ogawa 1974; Isaia and Masoni 1976; McWilliams and Potts 1978), which have been shown to affect uptake of waterborne contaminants such as methylmercury (Rodgers and Beamish 1983). Although metabolic rate significantly affects ^{137}Cs elimination in poikilotherms (Mailhot et al. 1989), little is known of the potential interactions of water chemistry and temperature on ^{137}Cs accumulation in fish.

In our experiments, we examined the effects of water chemistry, particularly K^+, on ^{137}Cs accumulation in rainbow trout. In our first set of experiments, we determined the effects of K^+ (0.08–0.31 mmol/L) and temperature (8 and 16°C) on the elimination of dietary ^{137}Cs. As we were unable to detect a significant effect of K^+ in our first experiments, our second set of experiments determined the effects of a much wider range of K^+ (0.01–3.4 mmol/L) on the both uptake (assimilation) and elimination of dietary ^{137}Cs. As these treatments also affected the concentrations of ions other than K^+, we also examined the effects of Na^+ and Ca^{2+} on ^{137}Cs dynamics. Steady-state models were then employed to investigate the potential environmental consequences of the observed effects.

Materials and methods

Fish and holding facilities

Juvenile rainbow trout (Ganaraska stock; initial weight ≈5 g) were supplied by the Ontario Ministry of Natural Resources' Normandale fish hatchery. Trout were held in tanks (70 L) that were initially supplied with a continuous flow of aerated groundwater (11.5 ± 0.5°C), then provided with the experimental water flow as described below. Throughout the experiments, the water in each tank was aerated and concentrations of dissolved oxygen were always greater than 80% of air saturation. Water flow was sufficient that the 90% water replacement time in each experimental tank (≈9 h) was within the guidelines recommended by Sprague (1969) for continuous flow bioassay systems. Photoperiod was maintained at 16 h of light with a gradual change of intensity for 0.5 h at each end of the light period. The fish within the tanks were fed a commercial trout diet, once daily, at the rate of 1.5% of the live weight. Meal size was calculated from the total live weight of all fish in the tank and adjusted every 14 d. This feeding regime was maintained throughout the experiments, except for the acclimation period preceding the second experiment. At least 3 weeks prior to an experiment, 5 of the 10 fish in each tank were anaesthetized (tricaine methane sulphonate at 50 mg/L) and individually marked by suturing a tag to the dorsal musculature. The mean live weight of the trout at the start of the first and second experiment was 15.7 ± 0.6 and 7.4 ± 0.2 g (mean ± SE), respectively. During the experiments, both total mortality and mortality in each tank was always less than 10%.

Experimental protocols

For both experiments, treatments were randomly assigned among the 16 experimental tanks. In the first set of experiments, the treatments were K^+ concentration (0.08, 0.15, 0.23, and 0.3 mmol/L; 3–12 mg/L) and temperature (8 and 16°C), with two tanks (replicates) assigned to each treatment combination. Potassium concentration was increased above the background K^+ concentration of Guelph groundwater (0.08 mmol/L) by mixing groundwater from temperature controlled headtanks with concentrated solutions of potassium bicarbonate ($KHCO_3$) using proportional diluters. The drip rates of the proportional diluters were monitored at least three times a day. K^+ concentrations were measured regularly and were consistently within 5% of nominal values. As the groundwater supplied was common to all tanks, total hardness (400 mg/L as $CaCO_3$) and other water quality parameters were the same among treatments. A detailed analysis of the Guelph groundwater conducted during this study indicated that the chemical characteristics of the water were the same as those reported previously (Dixon and Sprague 1981).

The trout were held under these experimental treatments for 14 d and then fed the ^{137}Cs labelled food for 5 d at

1.5% of live weight/d (all food was consumed). The fish were then fed unlabelled commercial food (at 1.5% of live weight/d) until the end of the experiment. During and following the feeding of labelled food, the water in tanks remained at the background levels of radioactivity. Retention of ^{137}Cs in the tagged fish was measured by regular whole body gamma counting from three days after the last feeding of labelled food to the end of the experiment (70 d).

In the second set of experiments, the range of K^+ concentrations was expanded (0.01–3.1 mmol/L ≡ 0.5–134 mg/L). The temperature in all tanks was maintained at 16±0.5°C, and the pH of all treatment conditions was 7.5±0.1. To attain the lower concentrations of K^+, the bulk of ions were removed from the groundwater by reverse osmosis (R/O). The R/O water was mixed with groundwater in a ratio of 9:1 in a temperature-controlled headtank, then mixed using proportional diluters with concentrated solutions of either potassium bicarbonate, sodium bicarbonate ($NaHCO_3$), or calcium chloride ($CaCl_2$) and added to each experimental tank. The drip rates of the proportional diluters were monitored at least three times a day, and the concentrations of K^+, Na^+, and Ca^{2+} measured regularly. The experimental treatments and measured ion concentrations are summarized in Table 1. There were three treatments with low concentrations of Na^+ (0.4 mmol/L ≡ 10 mg/L) and Ca^{2+} (0.3 mmol/L ≡ 10 mg/L) and K^+ concentration of 0.01, 0.3, and 3.4 mmol/L (0.5, 12, and 134 mg/L, respectively); one treatment with high Na^+, (2.9 mmol/L ≡ 68 mg/L), low Ca^{2+} (0.3 mmol/L), and low K^+ (0.01 mmol/L); one treatment with low Na^+ (0.5 mmol/L ≡ 12 mg/L), high Ca^{2+} (2.9 mmol/L ≡ 115 mg/L), and low K^+ (0.08 mmol/L); and one treatment in which K^+ was added (0.3 mmol/L) to Guelph groundwater (Na^+, 1.3 mmol/L ≡ 30 mg/L; Ca^{2+}, 2.6 mmol/L ≡ 105 mg/L). The highest concentration of K^+ was roughly one-third that of sea water and was approximately 15% of the concentration of $KHCO_3$, which was toxic to these rainbow trout in a preliminary acute lethality test (96 h). The molar concentrations of the high Ca^{2+} and Na^+ treatments were approximately equivalent to the highest K^+ concentration, and the treatment with K^+ at (0.3 mmol/L) added to Guelph groundwater provided a base for comparison with the first experiment.

In the second set of experiments, ion concentrations were gradually changed to treatment conditions over a 10-day period. Acclimation of the trout to the treatments was then monitored by the fish's appetite. Once daily, trout in each tank were fed as much of the unlabelled diet as they would consume within a 5-min interval, and food consumption was measured. The fish were assumed to have acclimated when the average consumption during 3 consecutive days varied less than 10% of the mean. All trout were acclimated in 2 weeks, with no apparent difference in food consumption among treatments. Following acclimation, the trout were fed the ^{137}Cs-labelled diet for 5 d at 1.5% of live weight/d (all food was consumed), then returned to unlabelled diet at 1.5% of live weight/d. During and following the feeding of labelled food, the water in tanks remained at the background levels of radioactivity. From 3 days after the last feeding of labelled food to the end of the experiment (70 d), retention of ^{137}Cs was determined by regular whole body gamma counting of tagged fish.

Table 1. Measured concentrations of potassium, sodium, and calcium in experimental tanks during the second experiment (16°C).

Treatment	No. of replicate tanks	K^+ (mmol/L)	Na^+ (mmol/L)	Ca^{2+} (mmol/L)
1	3	0.01±0.001	0.43±0.02	0.30±0.03
		(21)	(21)	(3)
2	3	0.34±0.01	0.41±0.01	0.32±0.02
		(20)	(21)	(3)
3	3	3.4±0.1	0.40±0.03	0.27±0.01
		(18)	(21)	(13)
4	2	0.01±0.002	2.9±0.08	0.26±0.01
		(19)	(19)	(9)
5	2	0.08±0.002	0.5±0.02	2.9±0.07
		(18)	(19)	(12)
6	3	0.32±0.01	1.3±0.03	2.6±0.04
		(21)	(21)	(21)

Note: Values are means ± SE. Values in parentheses are the number of measurements.

Radiological and analytical procedures

The ^{137}Cs-labelled diet was prepared by mixing aqueous ^{137}Cs chloride (specific activity of 3206 MBq/mg Cs; Amersham Canada Limited, Oakville, Ontario) with a powdered commercial trout food. The mixture was then extruded, lyophilized to 10% moisture and frozen (−20°C) in plastic bags until use. The 137Cs activity of the diet was ≈37 kBq/g, and the stable Cs^+ concentration in the diet (<1.3 μg/g) was not significantly altered by addition of the ^{137}Cs.

Retention of ^{137}Cs was monitored by whole body gamma counting of tagged fish using procedures similar to those of Rodgers and Beamish (1981). Briefly, the fish were placed in an acrylic cylinder filled with uncontaminated water that was centered between two rectangular detectors (NaI(Tl), 5 × 5 × 15 cm). The combined output from both detectors (Harshaw detectors and electronics) was summed and recorded. Three consecutive 1 min counts were made of each tagged trout at 3, 7, 14, 28, 42, 56, and 70 d after the last ^{137}Cs labelled meal was fed. Consecutive counts always varied by less than 5% of the mean of three counts. ^{137}Cs in water, diet, and homogenized fish tissue was measured using an automated gamma counter with a 7.5 cm diameter, 7.5 cm high, well-shaped NaI(Tl) crystal (Nuclear Chicago model 4233).

Phantoms of known activity and similar geometry to that of the fish were used as reference standards to interrelate the activity of fish and diet samples. The phantoms also provided a check on the efficiency of the detector, which was consistently 2%. For the size of fish used, relative counting efficiency was apparently independent of fish size, as the ratio of counts from the fish in the whole body gamma counter to that of homogenates of these fish was not significantly related to fish weight (6.4–24.2 g). Average initial ^{137}Cs activity for trout of the first and second experiment was 1470 ± 111 and 2005 ± 164 kBq/kg

Fig. 1. Average rate constants for the elimination of ^{137}Cs from rainbow trout (mean ± SE) in relation to temperature (8 or 16°C) and potassium concentration (0.08–0.31 mmol/L) of the water.

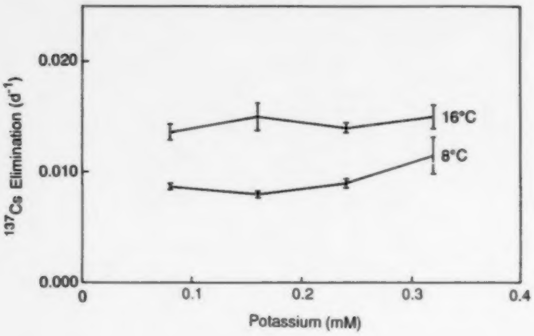


Fig. 2. Average rate constants for the assimilation of ^{137}Cs from diet by rainbow trout (mean ± SE) in relation to concentrations (mmol/L) of potassium (K^+), sodium (Na^+), and calcium (Ca^{2+}) in the water. Treatments of high Ca^{2+} concentration (>2.5 mmol/L) are indicated by hatched bars. Open bars indicate treatments of low nominal total hardness (0.3 mmol/L).

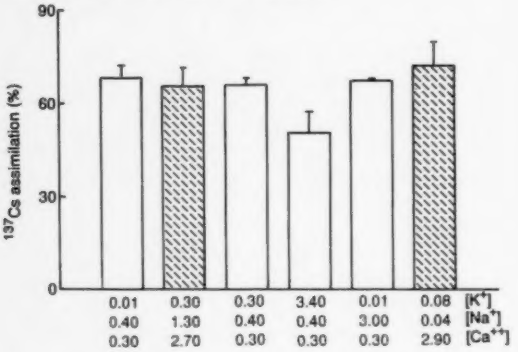
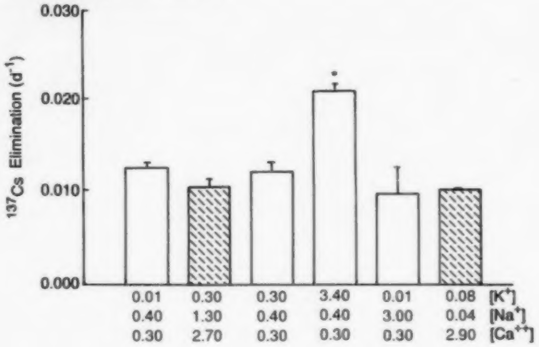


Fig. 3. Average rate constants for the elimination of ^{137}Cs from rainbow trout (mean ± SE) in relation to concentrations (mmol/L) of potassium (K^+), sodium (Na^+), and calcium (Ca^{2+}) in the water. Treatments of high Ca^{2+} concentration (>2.5 mmol/L) are indicated by hatched bars. Open bars indicate treatments of low nominal total mhardness (0.3 mmol/L). Treatments which are significantly different from other treatments are indicated by an asterisk.



live weight, respectively, and measured activity was always greater than 10 times background.

Potassium and sodium concentrations of water and serum were measured using a routinely calibrated flame photometer (Radiometer FLM 2). Calcium and total hardness (as $CaCO_3$) of the water were measured by titration with disodium ethylenediamine tetraacetate (EDTA) (APHA–AWWA–WPCF et al. 1989).

Statistical analysis

^{137}Cs retention in fish has been demonstrated to fit well to a two compartment first-order model, in which ^{137}Cs eliminated in the first or fast compartment is largely composed of non-assimilated ^{137}Cs, and the second or slow compartment represents "assimilated" ^{137}Cs, which is incorporated into the tissues of the fish (Häsänen et al. 1967; Gallegos and Whicker 1971; Kolehmainen 1972). In rainbow trout, the fast component does not contribute significantly to ^{137}Cs activity within 3 days after feeding labelled diet (Häsänen et al. 1967; Gallegos and Whicker 1971). Accordingly in both experiments, rate constants for elimination of assimilated ^{137}Cs (λ, d^{-1}) in each tagged trout were calculated as the slopes of a linear regression of the natural log of ^{137}Cs activity and time. All regressions were significant ($R^2 > 0.80$; $p < 0.05$). Biological half-lives ($Tb_{1/2}$) were calculated for each trout by dividing ln 2 by λ. Weight was not used as a covariate in this analysis because the relationship between elimination and change in weight during experimentation was not significant ($R^2 < 0.25$; $p > 0.05$). In the second experiment, assimilation of ^{137}Cs was determined for each experimental unit (tank) from the ratio of total ^{137}Cs activity of all trout (tagged and untagged) in the tank on day 3 after feeding labelled food, to the total ^{137}Cs activity in the food ingested by the fish during the 5-day exposure period.

The mean responses of the group of trout in a tank ($n = 16$) were analyzed by analysis of variance using two and one way models, for the first and second experiments, respectively. In the second experiment, preplanned comparisons were made using contrasts to further investigate treatment effects. The significance level for all tests was 5%. In all analyses, inspection of normal probability and residual plots indicated that assumptions of normality, homogeneity of variance, and independence were appropriate.

Results

In the first experiment, elimination of ^{137}Cs was significantly affected by temperature but not K^+ concentration (Fig. 1). Average elimination rate constants (mean ± SE; $n = 8$) and biological half-lives of trout at 16°C (0.014 ± 0.001 d^{-1} and 50 ± 2 d, respectively) were almost double those of fish at 8°C (0.009 ± 0.001 d^{-1} and 77 ± 5 d, respectively). However, elimination rate constants did not differ significantly over the range of K^+ concentrations (0.08–0.3 mmol/L) at either temperature.

In the second experiment, potassium concentration (0.01–3.4 mmol/L) significantly affected elimination but not assimilation of ^{137}Cs. Assimilation of dietary ^{137}Cs did not differ significantly among the treatments of K^+, Na^+, Ca^{2+}, and total hardness (Fig. 2), with an overall mean assimilation efficiency of 65 ± 3%. The rate of ^{137}Cs elimination at the highest K^+ concentration (3.4 mmol/L) was significantly greater than all other treatments (Fig. 3). Average elimination rate constants and biological half-lives were $0.021 \pm 0.001\ d^{-1}$ and 33 ± 1 d ($n = 3$), respectively, for trout exposed to the highest K^+ concentration, and $0.011 \pm 0.001\ d^{-1}$ and 63 ± 4 d ($n = 13$), respectively, for all other groups of fish. The rate of elimination of ^{137}Cs did not differ significantly among fish maintained at the lower concentrations of K^+ (0.01–0.3 mmol/L). Similarly, the rate of elimination of ^{137}Cs of fish maintained under the high Na^+ and Ca^{2+} treatments did not differ significantly from other treatments, where K^+ concentrations were 0.03 mmol/L or less. The rate of ^{137}Cs elimination in trout kept in Guelph groundwater with added K^+ (0.31 mmol/L) also did not differ significantly from other treatments where K^+ concentrations were 0.03 mmol/L or less; further this rate was not significantly different from that observed in the same treatment in the first experiment.

Discussion

Elimination of ^{137}Cs was significantly affected by K^+ and temperature, but assimilation of ^{137}Cs in rainbow trout was largely independent of the chemical quality of water and did not differ over the range of concentrations of K^+, Na^+, and Ca^{2+} tested. These results thus demonstrate that K^+ would influence accumulation of ^{137}Cs by affecting elimination and not assimilation, although the magnitude of this effect must also be evaluated. The trout were within their zone of ionic regulation, as in the second experiment, serum concentrations of K^+, and Na^+, serum osmolality, whole body concentrations of K^+, Na^+, and Ca^{2+}, and the Na^+–K^+ ATPase activity of gill filaments did not differ significantly among treatments (Cocchio 1993).

Although elimination of ^{137}Cs in trout was affected by K^+, the effect was apparent only at the highest K^+ concentration (3.1 mmol/L, ≈1/3 seawater), with no significant effect discernible over the lower range of K^+ (0.013–0.31 mmol/L). If K^+ serves essentially as a non-isotopic carrier for ^{137}Cs, elimination of ^{137}Cs would be expected to decrease over the range of K^+ concentrations, paralleling the reduction in K^+ flux observed in rainbow trout at lower concentrations of K^+ (Sanders and Kirschner 1983; Eddy 1985). Our inability to detect significant changes in ^{137}Cs elimination at lower concentrations of K^+ may indicate that K^+ flux is not completely analogous with that of ^{137}Cs. This conclusion is consistent with recent observations of "discrimination" in fish with respect to transport of K^+ and the larger alkali metals. Gardaire et al. (1991) indicated that for rainbow trout adapted to freshwater, uptake of K^+ occurs across a specific "potassium channel" on the secondary lamellae of the gill, and that transport of K^+ across this "channel" was competitively inhibited by alkali metals according to the sequence $K^+ > Rb^+ > Cs^+ > Na^+$ and Li^+. Similarly, Sanders and Kirschner (1983) noted that the gills of seawater adapted rainbow trout and sculpin (*Leptocottus armatus*) were ≈1.3 times more permeable to K^+ than ^{86}Rb. If this "discrimination" between K^+ and Cs^+ is general, application of the concept of K^+ as a non-isotopic carrier for understanding the environmental behaviour of ^{137}Cs may be severely constrained. In this respect, Kolehmainen (1972) noted a twofold variation in the concentration ratios of ^{137}Cs to stable cesium for different species of fish in White Oak Lake, while the concentration ratios of ^{40}K to stable potassium were rather constant. Similarly, Zach et al. (1989) concluded that levels and transfer of ^{137}Cs and ^{40}K were not closely related in a boreal terrestrial environment.

To further assess the consequences of the observed differences in rates of elimination of ^{137}Cs, we have estimated the steady state concentrations of ^{137}Cs which would apply to fish of the same mass (m), receiving the same input of ^{137}Cs, but eliminating ^{137}Cs at different rates. If the fish is represented as a single compartment system, the quantity of ^{137}Cs in the fish (q, Bq) at steady state may be described by the equation

$$[1] \qquad q = \frac{R}{\lambda} \text{ or } R = q\lambda$$

where R is the rate of ^{137}Cs uptake (Bq/d) and λ is the first-order elimination rate constant (d^{-1}) as determined in this study (Whicker and Shultz 1982). Equation 1 may also be rearranged as

$$[1a] \qquad R_L = q_L\lambda_L \text{ and } R_H = q_H\lambda_H$$

where q_L, R_L, and λ_L and q_H , R_H, and λ_H represent the above terms under conditions of low (L) and high (H) concentrations of K^+, respectively. To evaluate the effect of changes in elimination rate independently of changes in uptake rate, we also assumed that the rate of ^{137}Cs uptake would be equivalent at both concentrations of K^+ (i.e., $R_L = R_H$). Thus,

$$[2] \qquad q_L\lambda_L = q_H\lambda_H \text{ or } q_L = \frac{\lambda_H}{\lambda_L}q_H$$

and for $\lambda_H = 0.021$ ($K^+ = 3.4$ mmol/L) and $\lambda_L = 0.011$ ($K^+ \leq 0.3$ mmol/L)

$$[2a] \qquad q_L = \frac{0.021}{0.011}q_H \approx 2q_H$$

At steady state, the quantity of ^{137}Cs in a fish eliminating ^{137}Cs at the slower rate (λ_L) would thus be roughly twice that of a fish eliminating ^{137}Cs at the faster rate (λ_H). As we have assumed the fish were of the same mass (m), the concentration of ^{137}Cs in the fish in low K^+ water (q_L/m) would also be roughly twice that of a fish in high K^+ water (q_H/m). Consequently, these K^+ related changes in ^{137}Cs elimination would account for only an approximate doubling in concentration ratios (the ratio of ^{137}Cs concentration in the fish to water). As concentration ratios observed in wild fish vary by roughly three orders of magnitude, it would appear that the direct effects of K^+ on ^{137}Cs elimination in fish, by itself, can explain only a small fraction of this variability.

Alternative approaches such as the equilibrium food chain model of Thomann (1981) may be of greater utility.

In Thomann's model, the ^{137}Cs body burden in top predators was attributed to a combination of biomagnification of ^{137}Cs through food chain transfer, and initial salinity-dependent phytoplankton adsorption. Within waterbodies, species-specific differences in diet and food consumption, growth rate, and habitat use are also critical to the observed differences in ^{137}Cs accumulation among fish species (Forseth et al. 1991; Ugedal et al. 1994).

The constancy of assimilation of ^{137}Cs in rainbow trout exposed to a wide range of water chemistry indicates that K^+ would affect accumulation of ^{137}Cs by affecting elimination rather than uptake. The observed mean assimilation efficiency of ≈65% is similar to the value (65.9%) reported by Forseth et al. (1992) for brown trout fed fish muscle. However, both Forseth et al. (1992) and Kolehmainen (1972) observed large variations in assimilation efficiency among different diets with values ranging from <20% to >80%. In particular, the large quantities of sediment and particularly clay in the gut of benthic animals such as Ephemeropteran larvae may significantly reduce assimilation efficiency (Kolehmainen 1972). Variations in assimilation efficiency of this magnitude will significantly affect the body burden of the contaminant attained by the fish (Rodgers 1994) and may be another key factor in explaining differences in ^{137}Cs accumulation among fish species.

The increase of the mean ^{137}Cs elimination rate constant from 8 to 16°C corresponds to a Q_{10} of ≈1.7. As a Q_{10} of ≈2 is typical of a number of metabolic processes (Prosser 1973), these results are consistent with the hypothesis that ^{137}Cs elimination is a function of metabolic rate. The present results also agree with previous studies of the effects of temperature on ^{137}Cs elimination in rainbow trout (Gallegos and Whicker 1971) and brown trout, *Salmo trutta* (Ugedal et al. 1992), and support the general conclusion that ^{137}Cs elimination in poikilotherms is a function of metabolic rate (Mailhot et al. 1989). ^{137}Cs elimination in fish may thus differ significantly from some other contaminants, such as methylmercury and PCBs, where elimination is apparently independent of temperature and, by inference, metabolic rate (Sharpe et al. 1977; Norstrom et al. 1976). In nature, where temperature would affect ingestion of ^{137}Cs as well as elimination rate, the net effect of temperature on ^{137}Cs accumulation would depend on the relative magnitude of these changes (Kolehmainen 1974, Forseth et al. 1992).

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Predator avoidance in naive and experienced juvenile chinook and coho salmon

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Abstract: We compared the vulnerability of naive and experienced coho and chinook salmon to predation by rainbow trout and related these findings to observations of the specific behaviour of each species during an encounter with the predator. Naive chinook were significantly less vulnerable to capture than naive coho in an open field encounter but there was no difference in capture rate of experienced coho and chinook. In videotaped encounters we found that coho and chinook behaved in significantly different ways when attacked by the predator, both when naive and when experienced. Our results suggest that predator avoidance strategies are highly species specific, that both species adjusted their behaviour after experience with the predator, and that the behavioural changes adopted by coho improved their ability to avoid the predator in an open field but those adopted by chinook did not. Considerable recent research has explored the willingness of different species, sexes, or life stages to risk exposure to a predator to obtain some reward (usually food). Our results further suggest that published experiments that purport to demonstrate differences in risk taking among types of prey may, instead, demonstrate differences in predator avoidance capability.

Résumé : Nous avons comparé la vulnérabilité à la prédation par la truite arc-en-ciel chez des saumons cohos et quinnats novices et expérimentés, et mis ces résultats en rapport avec des observations du comportement spécifique de ces poissons pendant une rencontre avec le prédateur. Les quinnats novices étaient de façon significative moins vulnérables à la capture que les cohos novices lors d'une mise en présence dans un milieu ouvert, mais il n'y avait pas de différence dans le taux de capture des saumons et des quinnats expérimentés. Lors des rencontres filmées sur vidéo, nous avons observé que le coho et le quinnat se comportaient de façon notablement différente lorsqu'ils étaient attaqués par le prédateur, que les poissons soient novices ou expérimentés. Nos résultats indiquent que les stratégies d'évitement du prédateur sont fortement liées à l'espèce, que les deux espèces ont adapté leur comportement après avoir fait l'expérience du prédateur, et que les changements comportementaux adoptés par les cohos amélioraient leur aptitude à éviter le prédateur dans un milieu ouvert, ce qui n'était pas le cas pour les quinnats. D'importantes recherches ont récemment examiné dans quelle mesure des poissons, selon leur espèce, leur sexe ou leur stade vital, sont prêts à s'exposer à un prédateur pour obtenir une récompense (généralement de la nourriture). Nos résultats indiquent de plus que les expériences publiées qui prétendent démontrer des différences entre les types de proies dans la façon de prendre des risques révélaient plutôt des différences dans leur capacité d'évitement des prédateurs.

[Traduit par la Rédaction]

Introduction

The behaviour of prey animals facing a threat of being caught by a predator has been the subject of much recent research (reviewed in Lima and Dill 1990; Godin 1990). Several workers have shown that even closely related groups of animals may differ in their willingness to expose themselves to a predator and have interpreted these differences in terms of the natural histories of their subjects. In fishes, for example, differences in apparent risk-taking behaviour have been linked to species-specific feeding strategies (Magnhagen 1988; Abrahams and Healey 1993) or sex-specific feeding and breeding strategies (Abrahams and Dill 1989; Holtby and Healey 1990).

The willingness of an individual prey organism to expose itself to a predator must reflect the prey's assessment of the benefits to be gained (e.g., increased food intake) and the costs (e.g., the probability that it will be attacked and perhaps killed). Experimental designs to examine the risk taking in prey usually involve varying either the benefit or the cost side of this equation (Magnhagen 1988; Abrahams and Dill 1989; Abrahams and Healey 1993).

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Where different species, sexes, or morphs are being tested, however, any conclusion about relative levels of risk aversion from these experiments is premature unless the vulnerability (that is, the actual probability of being captured) of the prey types in the experimental situation is known to be equal. Since risk is, formally, the combination of the severity of an event with its probability of occurrence (Rowe 1986), a prey that swims almost into the mouth of a predator to obtain food may be accepting little risk if its ability to evade the predator virtually ensures that it will not be eaten. Similarly, a prey that is less willing than any other to expose itself to a predator may not be more risk averse than the others if its probability of being captured is much higher.

In fishes, relatively little research has been done on either the vulnerability of particular prey to a predator or the detailed behavioural responses that underlie vulnerability. Reist (1983) showed that morphs of brook sticklebacks (*Culea inconstans*) differed in their vulnerability to pike (*Esox lucius*) and adopted somewhat different behaviour in the presence of the pike. Webb (1982) showed that fathead minnows (*Pimephales promelas*) responded differently to attacks by different predators. Thus, it is reasonable to suppose that even closely related prey may differ in their vulnerability to a predator and may behave differently in the presence of a predator. In a recent study, Abrahams and Healey (1993) showed that juvenile coho salmon (*Oncorhynchus kisutch*) were less willing to expose themselves to a predator and also less vulnerable to capture in a standardized test than chinook salmon (*O. tshawytscha*), suggesting that coho were considerably more risk averse than chinook. Conclusions about relative levels of risk aversion among prey types that are not based on knowledge of prey vulnerability (Magnhagen 1988; Abrahams and Dill 1989) are, however, cast into doubt by these observations.

In the present study, we investigated differences in behaviour associated with differences in vulnerability to predators in juvenile coho and chinook salmon. Our objectives were to measure the relative vulnerability of juvenile coho and chinook salmon to predation in a standardized test and to relate these findings to specific aspects of their behaviour during an encounter with a rainbow trout (*O. mykiss*, formerly *Salmo gardnerii*). As earlier studies have shown that vulnerability decreases after encounters with predators (Kanid'yev et al. 1970; Ginetz and Larkin 1976; Patten 1977; Olla and Davis 1989), we conducted our experiments on both naive and experienced juvenile salmon. Following Abrahams and Healey (1993), we expected that coho would be less vulnerable to the predator than chinook salmon and that their differences in vulnerability would be reflected in species specific behavioural responses to the predator.

Materials and methods

The fish used as prey in the experiments were hatchery-reared salmon parr from the Vedder River (coho), Stuart River (chinook), and Capilano River (both species) stocks in British Columbia. We obtained these prey from Canadian Department of Fisheries and Oceans hatcheries at Cultus Lake and Capilano River. Individual prey ranged in size from 52 to 112 mm fork length depending on the experiment, species, and time of year. During any particular experiment the prey differed in size by only a few millimetres. The rainbow trout predators (fork length, 33–35 cm) were from a commercial hatchery and were trained to feed on juvenile salmon for this study. We transferred all fish to aquaria at the University of British Columbia in April and held them at ambient temperatures, ranging from 9 to 14°C, throughout the study (April–August 1991). We fed prey a diet of commercial fish pellets and predators a diet of surplus prey and commercial fish pellets.

Experiment 1

This experiment was designed to test two hypotheses about the vulnerability of naive and experienced coho and chinook.

(*i*) Coho are less vulnerable than chinook to a rainbow trout predator. This hypothesis derives from Abrahams and Healey's (1993) result. We tested this hypothesis by presenting pairs of naive coho and chinook or experienced coho and chinook to a predator as described below.

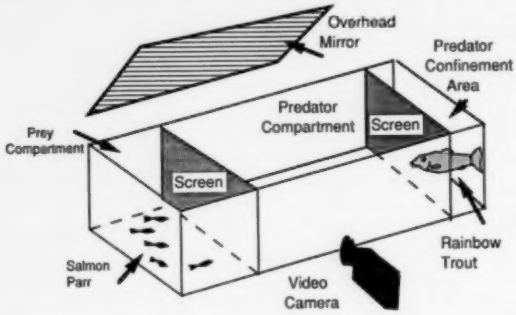
(*ii*) Experienced coho and chinook are less vulnerable to a predator than naive. This hypothesis derives from the literature demonstrating that experienced prey (including salmonids) are less vulnerable to predation than naive. We tested this hypothesis by pairing naive with experienced chinook and naive with experienced coho in encounters with a predator.

The design of the experiment was to release, simultaneously, a pair of prey (differing in species or experience with predation) into the centre of an oval 900-L fiberglass tank containing a rainbow trout. The tanks contained no refuges for the prey. We chose the members of each pair of prey at random from stock tanks of each species and marked one (randomly assigned) fish by clipping the adipose fin or by cutting a notch in the upper lobe of the caudal fin. Since the ability to avoid predators increases with size in salmon (Bams 1967; Patten 1977), we matched the prey for size. The two categories of prey used in a test had identical average sizes and no pair differed in length by more than 2 mm (max. 2.5% difference in size). We confined the prey in an opaque cylindrical tube in the experimental tank for at least 90 min prior to release to allow the waning of stress from handling (following results of Olla and Davis 1989). Following release of prey into the predator tank, we recorded the number of attacks on each prey, the time taken to capture the first prey, and which prey was captured first.

We used six different rainbow trout as predators in these experiments and presented an individual predator with prey no more frequently than once per day. During the experiments, predators received no food other than the prey presented during trials. We used individual prey only once. A single trial involved the release of one pair of prey to one of the predators. An individual test involved 8 or 9 trials with each predator (48–54 trials in total). Occasionally a trial failed because the predator did not attempt to capture the prey, and we discarded the results of the trial. For the purpose of statistical analyses, we assumed all trials were independent.

At the start of our tests none of the prey had ever encountered a potential predator and we considered them

Fig. 1. Experimental setup for experiment 2. For each trial, the screen separating the prey and predator compartments was raised. Then the predator confinement area was opened and a food pellet dropped below the screen separating predator and prey compartments. The predator darted toward the prey compartment to sieze the food pellet. The behaviour of salmon parr and the predator's simulated attack were filmed via the overhead mirror. See text for a more detailed description.



naive for the purposes of our experiments. To produce experienced fish we released a group of 30–40 prey into an aquarium that contained a trout predator and allowed the predator to attack the group until it had consumed two prey (this usually occurred within 2 min after the release of the prey). After this procedure was repeated twice, we considered the surviving prey fish to be experienced.

Experiment 2

The objective of this experiment was to test two hypotheses about the behaviour of salmon during an attack by a predator: (*i*) coho and chinook differ in their specific behavioural responses to the attack of a predator; and (*ii*) experienced chinook and coho differ from naive salmon in their response to the attack of a predator.

We tested these hypotheses by comparing the behaviour of each species and category (experienced or naive) of prey before, during, and after the attack of a predator.

We conducted these experiments in a rectangular aquarium 180 cm long by 115 cm wide by 30 cm deep (Fig. 1). The aquarium was divided into three sections by removable opaque partitions placed 60 cm from the left end and 20 cm from the right end. The walls and bottom of the aquarium were painted white and it was lit from above by two 350-W incandescent light bulbs.

The predator used in this experiment was a rainbow trout that had never learned to prey on juvenile salmon, despite repeated attempts to train it. This fish could be confined to the small right-hand compartment of the tank and was trained to dart toward the left-hand end to receive a pellet of food when released from confinement. To the group of juvenile salmon in the left hand compartment, this dart simulated a real attack. In this experiment a single trial consisted of the behavioural results collected during a single attack of the predator.

Via an angled mirror above the aquarium, we videotaped the behaviour of a small group of naive or experienced chinook or coho in the left-hand compartment of the tank to determine their response to the dart of the predator. One hour prior to a trial, we confined the predator in the right-hand compartment of the tank and introduced five to seven chinook or coho that had previously been acclimatized to the aquarium to the left hand compartment. At this time, a transparent screen separated the prey from the remainder of the tank. We started videotaping 30–60 min later, by which time several prey fish had usually begun to feed on the small amounts of food offered. About 1 min prior to the release of the trout, we raised the transparent screen separating the prey from the main compartment of the tank. At the moment the trout appeared in the exit of the right compartment, we dropped a food pellet into the water 60 cm from the left end of the aquarium. The predator would then dart toward the left end of the tank to capture the pellet, thus performing a realistic but benign attack toward the prey. We ended the videotaping about 1 min following the dart of the predator. Prey were used only once in these tests and each test consisted of between 10 and 12 trials. For statistical analysis we pooled the results of individual behaviour from all trials within a test.

From the videotapes we recorded the behaviour of each of the prey before any disturbance from raising of the screen (prior), 2 s prior to the predator's attack (−2 s), 1 s after the predator initiated its attack (+1 s), and 5 s after it initiated its attack (+5 s). We chose these time points to provide information on the behaviour of prey: (*i*) when undisturbed; (*ii*) following a disturbance in the tank but no predator was present; (*iii*) when the predator was capturing the food pellet; and (*iv*) a few seconds after the attack of the predator.

For each of the four time points we recorded six categories of prey behaviour: (*i*) holding still; (*ii*) feeding (i.e., searching for food on the bottom); (*iii*) wandering (slow swimming with many turns); (*iv*) swimming slowly (<1 body length per second, few turns); (*v*) swimming fast (>1 body length per second, few turns); and (*vi*) darting (rapid movement following a "c" or "j" shaped body flexure (Webb 1986)). In addition, we recorded the orientation of the prey that were holding still or moving slowly as (*i*) in the direction of travel of the predator ±45°; (*ii*) at right angles to the direction of the travel of the predator ±45°; (*iii*) opposite to the direction of travel of the predator ±45°. Other measurements included the distance from each prey fish to its nearest neighbour (one centre of body mass to the next) and the reaction distance of the first fish that showed a clear flight response to the predator (distance from centre of body of the prey to tip of the snout of the predator).

Results

Experiment 1

When presented as pairs to a hungry trout, significantly more naive coho than naive chinook salmon were caught first ($\chi^2 = 7.08$, df = 1, $p < 0.01$) and significantly more naive than experienced coho were caught first ($\chi^2 = 3.43$, df = 1, $p < 0.05$) (Table 1). Neither experienced coho and chinook nor naive and experienced chinook differed in their probability of first capture. The preferences of the

Table 1. Summary of observations on vulnerability of naive and experienced (exp.) coho and chinook in experiment 1.

Variable	Test 1 (*a*) Naive coho (*b*) Naive chinook	Test 2 (*a*) Exp. coho (*b*) Exp. chinook	Test 3 (*a*) Exp. chinook (*b*) Naive chinook	Test 4 (*a*) Exp. coho (*b*) Naive coho
Fish length, mm (SE)	80 (0.14)	92 (0.11)	80 (0.08)	65 (0.06)
No. of trials	51	51	45	42
"*a*" captured	35	26	24	15
"*b*" captured	16	25	21	27
Attacks to capture (adj.)	9.4	8.3	7.3	6.5
Time to capture (s, adj.)	25.5	17.0	16.8	12.8
When "*a*" captured, adj. attacks *a/b*	6.3/4.2*	7.4/2.4*	4.5/4.0	4.9/2.5
When "*b*" captured, adj. attacks *a/b*	2.3/6.3*	3.1/7.8*	2.6/5.5*	2.6/4.0

Note: Adjusted values represent the variable adjusted for fish size by ANCOVA. See text for explanation of other differences.

*Paired values that are significantly different.

six individual predators did not differ significantly within tests (χ^2 tests, all $p > 0.05$).

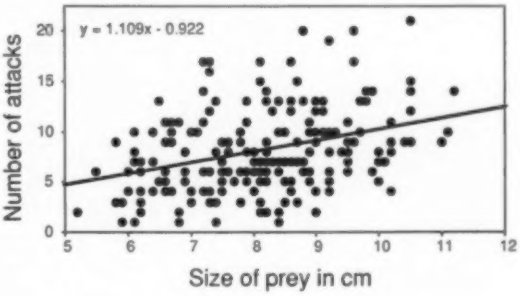
The average number of attacks until capture was positively correlated with the body size of the prey (Fig. 2). The experienced prey in the second test were captured after fewer attacks and in a shorter time than the naive prey in the first test, after adjustment of the data for prey body size (unpaired $t = 7.98$ for attacks and $t = 36.00$ for time, df = 100, both $p < 0.001$). Regardless of experience level, the smaller coho in the fourth test were caught faster and after fewer attacks than the larger chinook in the third test (unpaired $t = 14.83$ for attacks and 9.31 for time, df = 83, both $p < 0.001$) (Table 1). These results appear to reflect a combination of continued learning of the experimental set up by the trout as well as prey size effects.

In only 3% of the trials was the first attack on a prey successful. In 77% of the trials both fish in a pair were attacked. In most instances prey capture occurred after the predator focused its attention on one of the fish and hunted it down, an observation that is reflected in the greater number of attacks on the prey that was eventually captured (Table 1).

The predominant defensive behaviour of the prey in these experiments was to dart away upon an attack and to stay motionless between attacks with a preferred position near the surface and along the edges of the tank. If a predator interrupted its hunting activity for 20–30 s, some prey would start to swim and explore the tank.

The average time and the number of attacks until capture of a prey differed significantly among the six rainbow trout predators (average times to capture ranged from 12 to 21 s, $F = 4.32$, df = 5/10, $p < 0.001$; average number of attacks ranged from 6 to 10, $F = 3.3$, df = 5/201, $p < 0.05$). As all predators were presented with similar numbers of prey in each test, pooling of the data was still possible. However, variation among predators contributed to uncontrolled variation in the results.

Fig. 2. Number of attacks to capture a prey in experiment 1 in relation to the size of the prey. Pooled data from all four tests. The regression is significant at $p < 0.05$.

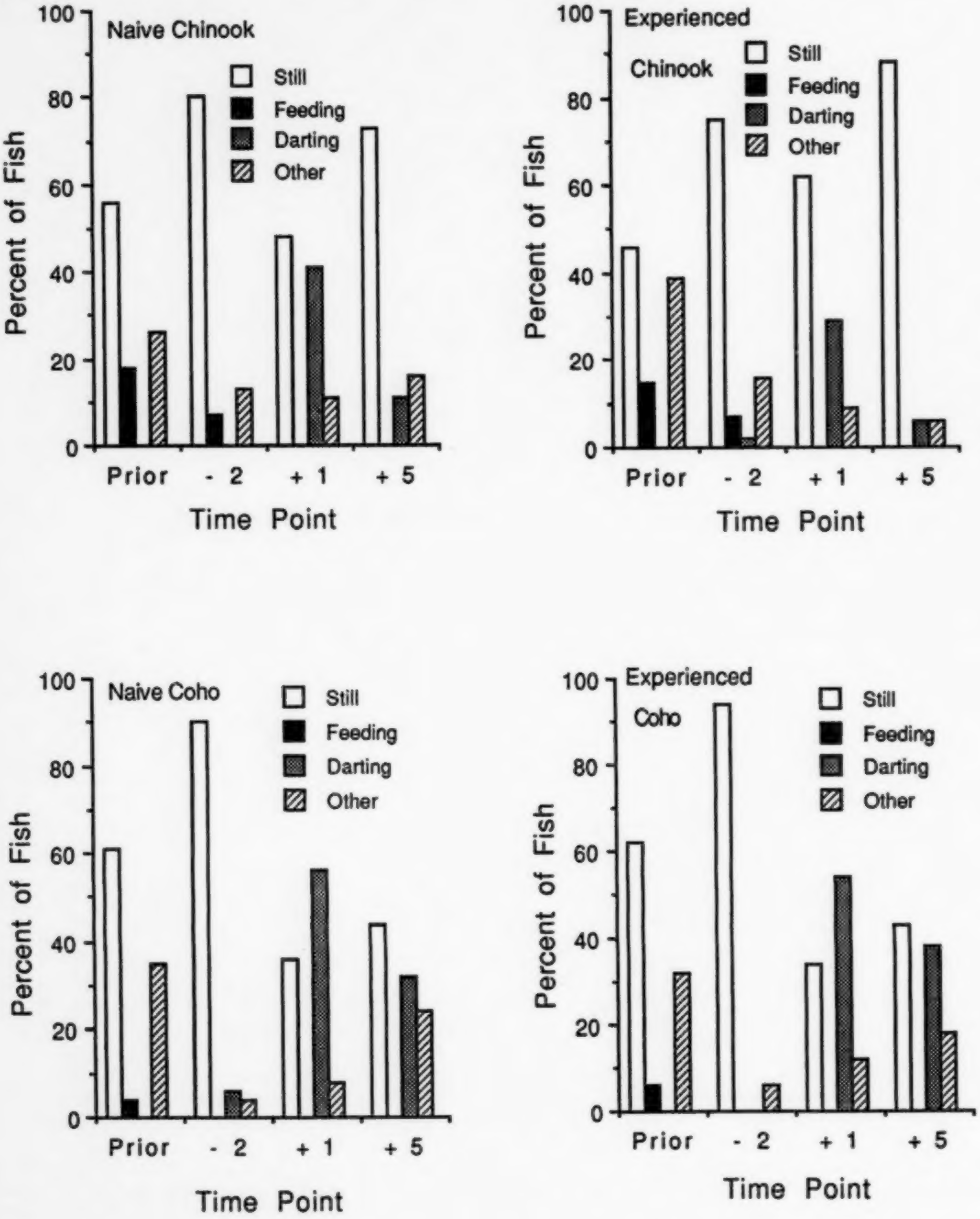


Experiment 2

The predominant behaviour of coho and chinook parr in the simulated predator attacks, at all time points, was to hold still (Fig. 3). Feeding and various types of swimming (wandering, slow, fast) were important only before the partition in the tank was raised. Flight was a characteristic behaviour during and, to a lesser extent, 5 s after the simulated attack of the rainbow trout (Fig. 3).

For naive chinook salmon, the frequency of the six behaviours changed significantly at each time point (all $\chi^2 > 11.9$, $p < 0.05$, Fig. 4) with the greatest change between time points −2 s and +1 s ($\chi^2 = 37.8$, $p < 0.001$). The behaviour of experienced chinook changed significantly when the transparent screen was raised and when the predator began its attack (both $\chi^2 > 17.0$, $p < 0.01$) but did not change between time points +1 s and +5 s (Fig. 4). The behaviour of naive chinook at time point +5 s differed significantly from their behaviour at both time points prior to the attack of the predator (χ^2 values both >17.8, $p < 0.01$), whereas the behaviour of experienced chinook at time point +5 s was similar to that at time point −2 s ($\chi^2 = 8.5$, $p >$

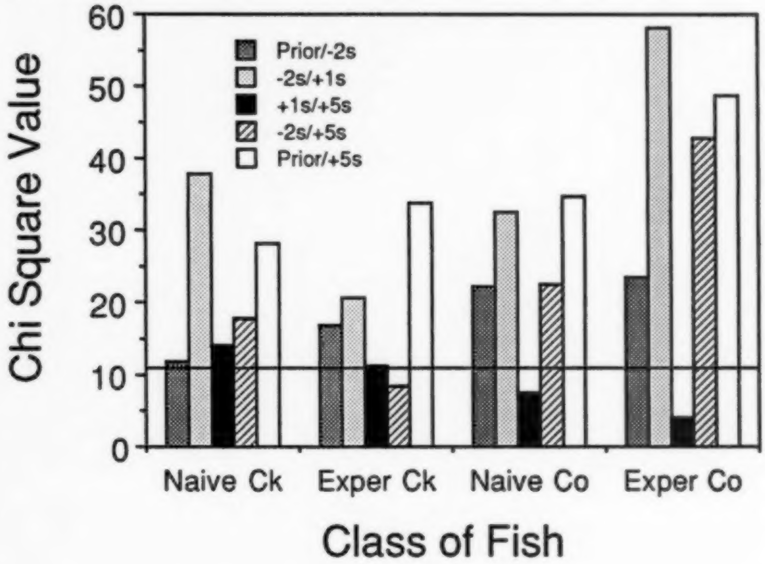
Fig. 3. Percentage of naive and experienced chinook and coho that were holding still, feeding, darting, or showing other forms of swimming (wandering, swimming slowly, or swimming fast) at time points prior, −2 s, +1 s, and +5 s during a predator attack in experiment 2. See text for fuller description of the time points.



0.05). These latter comparisons show that naive chinook continued to change their behaviour throughout the test period, whereas experienced chinook rapidly reverted to behaviour that we termed "vigilant" (see Discussion).

The behaviour of both naive and experienced coho changed significantly between time points prior and −2 s, and −2 s and +1 s (all $\chi^2 > 22.3$, $p < 0.001$) but not between +1 s and +5 s (Fig. 4). The greatest change was

Fig. 4. Chi square values for comparisons of the frequency of different behaviours between points in time within treatment groups in experiment 2. The horizontal line marks the significance threshold at $p = 0.05$. Bars that extend above that line indicate significant differences in behaviour between time points. Co, coho; Ck, chinook.



between time points −2 s and +1 s for both types of coho. The behaviour of coho at time point +5 s differed significantly from their behaviour at both time points before the predator's attack (Fig. 4). Changes in the behaviour of both naive and experienced coho between time points were dominated by changes in the numbers that were darting or still, although other types of swimming also changed in frequency when the partition was raised (Fig. 3).

Three comparisons between categories of fish for each time point were significant. At −2 s, naive coho and chinook differed in behaviour as did experienced coho and chinook (χ^2 values 13.7 and 11.5, respectively, $p < 0.05$). At +5 s, experienced coho and chinook differed in behaviour ($\chi^2 = 23.9$, $p < 0.001$). After raising the partition (−2 s), almost all coho became motionless, whereas some chinook continued to feed and display other types of swimming (Fig. 3). After the attack (+5 s), equal numbers of experienced coho were fleeing and motionless, whereas 90% of the experienced chinook were motionless (Fig. 3).

The orientation of the salmon relative to the direction of the predator also differed among species and with experience. During the attack, none of the groups showed a preferred orientation. At time points −2 s and +5 s, naive chinook and experienced coho tended to align toward the trout, whereas naive coho preferred an alignment at right angles (Fig. 5). Experienced chinook showed no preferred orientation.

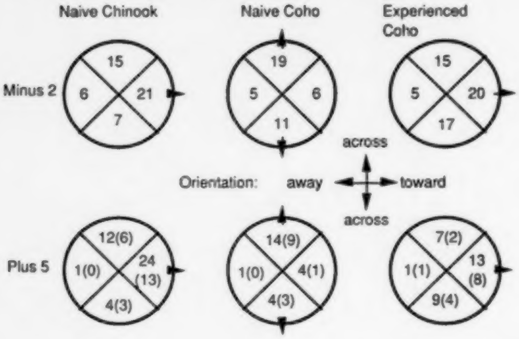
We transformed the minimum distances between members of each group of prey into body lengths to allow comparisons among the species (chinook fork length = 9.9 cm ±0.71 SD, coho = 6.8 cm ±0.44 SD). Since the vertical position of the fish in the 30 cm deep water column is unknown, the data contain an error that is potentially greatest in the shortest apparent distances between neighbours. The only significant difference in the distribution of the fish occurred after raising the partition; the experienced coho stayed closer together than the naive coho by about 1/2 body length (non-parametric Mann–Whithey test $Z = 2.21$, $p < 0.05$).

The speed of the attack by the trout varied from 0.55 to 1.5 m/s but was not significantly different among tests ($F = 0.198$, df 3/43, $p > 0.05$). There was no relationship between the speed of the trout and the reaction distance of the coho or chinook. Experienced chinook displayed the shortest reaction distance (average 41 cm, ±3.21) and experienced coho showed the greatest (average 66 cm, ±2.62). This difference was significant ($t = 3.18$, df = 13, $p < 0.01$) and translates into a delay of flight initiation in chinook by 0.25 s based on an average speed of the trout of 1 m/s.

Discussion

Both species and experience with a predator influenced the vulnerability of coho and chinook to a rainbow trout predator in our experiments but not in the way that we expected. Based on a considerable literature demonstrating improved predator avoidance after a brief experience with predation (Thompson 1966; Kanayama 1968; Ginetz and Larkin 1976; Volkova 1976; Patten 1977; Olla and Davis 1989) and Abrahams and Healey's (1993) finding that coho were less vulnerable than chinook to a predator, we expected that both species would be less vulnerable when experienced and that coho would be less vulnerable than chinook.

Fig. 5. The number of salmon orienting toward four sectors in the tank at time points −2 s and +5 s in experiment 2. The numbers in parentheses at time point +5 s represent the fish that changed their orientation from before to after a predator attack. Alignments at right angles to the predator were summed for the chi square analysis. Arrows on each circle indicate orientation significantly different from random. Data for experienced chinook are not shown as these fish did not prefer a particular orientation.



Instead we found that chinook were less vulnerable than coho and only coho appeared to benefit from experience. All published studies differ significantly in the design to measure vulnerability, however, which probably accounts for much of the variation in results among studies. For example, Abrahams and Healey's (1993) measure involved prey escaping to a refuge, whereas our design contained no refuge.

Also contrary to our expectation, we did not find any clear association between time or attacks to capture and vulnerability. Only prey size was significantly correlated with vulnerability. This was unexpected because it seemed to us that both time to capture and attacks to capture should be primary indicators of a prey's ability to avoid a predator. Our results may have been partially confounded by the continued learning of the predators but this factor was insufficient to account for the complete absence of a relationship.

More attacks were directed at the prey individual that was eventually captured in each trial. There appeared to be a point in each trial at which some subtle aspect of a prey attracted the predator's attention. Once this occurred, the predator directed most of its attacks at that individual until it was captured.

We conclude that several factors determine vulnerability in laboratory tests. The design of the experiment may favour tactics of one prey over another. We believe this accounts for the opposite results obtained by Abrahams and Healey (1993) and ourselves. Our design, without a prey refuge, represents the simplest possible habitat and probably limits the range of opportunities for successful anti-predator behaviour, whereas designs with prey refugia may permit more behaviours to come into play. Experience with predation influences vulnerability but the nature of that experience may also be important in determining prey behaviour (Gotceitas and Godin 1993). In our trials, the critical factor determining vulnerability appeared to be some characteristics (physical, behavioural) that caused the predator to focus its attention on a particular prey. The results of our more detailed behavioural observations illustrate some of the behavioural differences between chinook and coho that may account for differences in vulnerability.

All groups in experiment 2 clearly reacted to the dart of the rainbow trout. Even before the attacks, however, the fish reacted to the disturbance caused by raising the transparent partition. The behaviour observed in the course of these trials could, therefore, be classified into three broad categories: (*i*) undisturbed behaviour prior to raising the partition (feeding, social interactions, and slow swimming with many turns); (*ii*) vigilant behaviour after the partition was raised (primarily holding still); and (*iii*) flight during the rush of the predator. Undisturbed behaviour was similar in all treatment groups but at other time points the frequency of various types of behaviour differed among types of prey. Coho salmon seemed more sensitive to the disturbance from raising the partition and chose vigilant behaviour to a greater degree than chinook, many of which continued to show undisturbed behaviour. Coho also showed more flight behaviour than chinook and continued to display flight behaviour longer than chinook. Chinook tended to remain still during the predator's rush and, when they did flee, returned to vigilant behaviour soon after the attack (within 5 s).

Our assumption was that there was one optimal predator avoidance strategy for small salmon faced with a large piscivore in an open environment. Based on the results of experiment 1, in experiment 2 we expected to find big differences in behaviour between naive coho and chinook as well as between naive and experienced coho but small differences in behaviour between experienced coho and chinook as well as between naive and experienced chinook.

Consistent with our expectations, the behaviour of naive and experienced chinook did not differ significantly except in their orientation (naive chinook oriented toward the predator, whereas experienced chinook showed no preference). Naive and experienced coho differed in both orientation (naive coho oriented at right angles to the predator and experienced oriented toward the predator) and in their spacing (experienced coho forming closer groups). Naive coho and chinook differed in orientation and in their behaviour after the partition was raised. Contrary to expectation, however, experienced coho and chinook showed a number of differences in behaviour at time points +2 s and +5 s and in their reaction distance, experienced coho reacting sooner than chinook. We conclude, therefore, that coho and chinook may have different "optimum" avoidance strategies.

Different solutions to the problem of avoiding a piscivore predator suggest different optimal alignments toward the predator (Weihs and Webb 1984). None of the prey groups preferred an orientation away from the predator, which would maximize the distance from the predator (Weihs and Webb 1984; Hall et al. 1986). Instead, naive chinook and experienced coho preferred to face the threat. This may help the prey avoid detection by displaying the smallest diameter to the predator. Facing the predator also allows the prey to monitor the predator in its small binocular field of vision so that distance can be judged accurately and a flight response optimally timed. Such monitoring may be beneficial if the prey's main strategy to avoid capture is to out-manoeuvre the predator (Webb 1976). Experienced coho chose a different orientation from naive coho, which suggests that they fine tuned their defensive tactics based on their experience with the rainbow trout, whereas chinook gave up their preferred orientation after being exposed to a predator. The differences between the species and changes in orientation with experience suggest that there is no single preferred orientation toward a predator and orientation must be considered in the context of the defensive strategy of the prey in a particular situation.

Experienced coho formed closer groups than naive coho during vigilant behaviour. Ginetz and Larkin (1976) also noted that experienced sockeye salmon fry formed compact schools when they encountered a predator, whereas naive fry did not. Forming a school may serve as a defense by confusing the predator and diluting the predation effect (Cushing and Harden-Jones 1968, Hamilton 1971, Milinski 1977, Major 1978) and experienced coho may employ this strategy more than chinook.

If the prey's avoidance strategy is to maximize its distance from the predator, then the less vulnerable fish should react earliest. Naive chinook, however, did not react earlier than naive coho, whereas experienced coho showed a greater flight initiation distance than the equally vulnerable experienced chinook. Dill (1974) found that experience with a model predator increased the reactive distance of zebra danios (*Brachydanio rerio*), supposedly through earlier stimulus recognition.

The effect of experience was generally more evident in interspecific than intraspecific comparisons. Experienced fish of the two species differed in orientation, in reactive distance, behaviour at −2 s and +5 s as well as in their individual choice of behaviour, whereas naive fish differed only in their orientation and in their behaviour at −2 s. This suggests that experience may have aided in the expression of species-specific predator avoidance behaviour. Suboski (1988) argued that the defensive behaviour in fish must be inate and that experience with predators serves as a releasing stimulus for that behaviour because learning by trial and error is not feasible.

Our observations in experiment 2 indicate strong species specific differences in the response of coho and chinook to a predator. These differences presumably underlie the differences in vulnerability observed in experiment 1, but the specific causes of vulnerability remain unresolved. Changes in orientation might reduce the "detectability" of experienced coho over chinook, but the tendency of coho to continue flight behaviour longer than chinook should keep the detectability of experienced coho high. Overall, chinook behaviour suggests less agitation in the presence of the predator and a strategy based on minimizing detection through keeping still. Coho behaviour, on the other hand, suggests a strategy based on rapid and early flight with, perhaps, a dependence on manoeuvrability and school cohesion to avoid capture.

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Differences in the onset of salinity tolerance between juvenile chinook salmon from two coastal Oregon river systems

Karen Young Kreeger

Abstract: Trask River juvenile chinook salmon (*Oncorhynchus tshawytscha*) rear in an estuarine environment, whereas Rogue River juveniles rear in a riverine environment, suggesting these populations differ in the ontogeny of smolting physiology. To study differences in the onset of salinity tolerance between these populations, I reared individuals from the Trask and Rogue rivers (Oregon) in a common environment and monitored changes in their physiology throughout their first 5–6 months. Trask fish were able to tolerate oceanic salinities at a smaller size and younger age than Rogue juveniles. Fifty days post-first feeding and at <2 g 65% of Trask juveniles survived in 32 ppt compared with 40% of Rogue juveniles. Trask juveniles also maintained their water balance in 32 ppt seawater relative to freshwater at 50 days post-first feeding compared with 80 days for Rogue juveniles. Plasma sodium concentration indicated that both populations did not competently osmoregulate until approximately 108 days post-first feeding. Although the minimum size and age of salinity tolerance depended on which indicator of tolerance was chosen for study, physiological parameters suggested interpopulational differences in the timing of the onset of salinity tolerance.

Résumé : Les juvéniles de saumon quinnat (*Oncorhynchus tshawytscha*) de la rivière Trask grandissent dans un milieu estuarien, tandis que ceux de la rivière Rogue se développent dans un milieu lotique; on pourrait penser que ces populations diffèrent sur le plan ontogénétique dans la physiologie de la smoltification. Pour étudier les différences entre ces populations dans l'apparition de la tolérance à la salinité, j'ai élevé des spécimens de la Trask et de la Rogue (Oregon) dans un même milieu et j'ai surveillé leurs modifications physiologiques pendant les 5–6 premiers mois. Les poissons de la Trask étaient en mesure de tolérer des salinités océaniques à une taille plus faible et à un âge plus bas que les juvéniles de la Rogue. Cinquante jours après le début de l'alimentation, et à <2 g, 65% des juvéniles de la Trask ont survécu à 32‰, contre 40% des juvéniles de la Rogue. Les juvéniles de la Trask réussissaient aussi à maintenir leur bilan hydrique dans de l'eau de mer à 32‰ par rapport à l'eau douce 50 jours après le début de l'alimentation contre 80 jours chez les juvéniles de la Rogue. La concentration de sodium dans le plasma indiquait que chez ces deux populations l'osmorégulation n'était efficace que 108 jours environ après le début de l'alimentation. Alors que la taille et l'âge minimaux de tolérance à la salinité dépendaient de l'indicateur de tolérance choisi pour l'étude, les paramètres physiologiques indiquaient l'existence de différences entre les populations dans l'apparition de la tolérance à la salinité.

[Traduit par la Rédaction]

Introduction

The developmental process of smolting in salmonids has been a subject of research for nearly 60 years (e.g., Hoar 1939), yet little is clearly understood about the genetics and evolution of smoltification and life-history characteristics of juvenile salmonids. Among salmonids, chinook salmon (*Oncorhynchus tshawytscha*) show the greatest variation in juvenile life-history patterns (Reimers 1973; Carl and Healey 1984; Withler et al. 1987; Taylor 1988; Nicholas and Hankin 1988; Taylor 1990*a*, 1990*b*, 1990*c*). Chinook salmon migrate to sea over an extended period compared with the

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Table 1. Adult and juvenile life-history traits for Trask fall and Rogue spring chinook salmon.[a]

Trait	Trask fall	Rogue spring
	Juveniles	
Distribution	Mainstem, estuary through Sept.	251 km of mainstem below dam through Sept.
Length in estuary[b]	10.8–13.0 cm FL	10.1–12.0 cm FL
Age at ocean entrance	Not much known, possibly subyearling estuarine	Mostly subyearling riverine
Size at ocean entrance	Not available	9.11–11.7 cm FL
	Adults	
Distribution of ocean catch	North migrating	South migrating, Oregon and northern California
Timing of return and spawning	Enter Sept.–Feb., spawn Oct.–Mar.	Enter system Mar.–June, spawn above Gold Ray Dam Sept.–Nov.
Age and size at spawning	Mostly age 4–5 years: age 4, 90 cm; age 5, 100 cm	Age 2–5 years: age 4, 77 cm; age 5, 88 cm
Fecundity	Age 5 and 100 cm FL, 5500–6000 eggs	Age 4 and 75 cm FL, 3000–3700 eggs

Note: FL, fork length.

[a] Nicholas and Hankin 1988, and Oregon Department of Fish and Wildlife, 2501 SW First Ave., P.O. Box 59, Portland, Oreg., U.S.A., personal communication.

[b] Length measurements are from a coastal survey, conducted in Sept. 1978–1987 and probably include migrant fall and spring juveniles. Trask River measurements also include fish from the other four rivers that drain into Tillamook Bay.

short migratory windows of coho salmon (*O. kisutch*). Juvenile chinook gradually acclimate to high salinity through the tolerance of their tissues, not regulation by cellular organelles, as in chum (*O. keta*) and pink (*O. gorbuscha*) salmon (Weisbart 1968). Chinook salmon show two primary patterns of smoltification and migration; (*i*) "ocean-type" juveniles, which smolt and migrate to sea during the first summer after emergence, and (*ii*) "stream-type" juveniles, which reside in fresh water for 1–2 years before smolting and migrating (Carl and Healey 1984; Taylor 1988; Taylor 1990*b*, 1990*c*).

McCormick and Saunders (1987) suggested that smoltification should be considered "a developmental process subject to changes in timing during the course of salmonid evolution," thereby facilitating the formation of hypotheses and comparisons of the adaptive mechanisms governing seawater entry and tolerance. In this study, I extend McCormick and Saunders' idea that heterochrony (changes in the timing of development) has occurred in the evolution of differences in osmoregulation among salmonid species to compare differences in the timing of salinity tolerance among populations of chinook salmon. Indeed, Taylor (1990*a*) suggested that differences in migration distance and fresh water temperature regime may result in natural selection for different sizes at migration among chinook populations and might promote geographic variability in age and (or) size at seaward migration because of differences in growth opportunity between fresh water and ocean rearing environments.

Two populations of chinook salmon from coastal rivers in Oregon were used to test for phenotypic differences in the timing of the onset of salinity tolerance. The Trask River fall and Rogue River spring populations of chinook salmon were chosen because of their contrasting environmental regimes and corresponding adaptations in adult and juvenile life-history characteristics (Table 1). Trask juveniles, on the one hand, migrate from spawning beds up to 64 km from the mouth of Tillamook Bay, through a gentle gradient mainstem, and a large, well-mixed estuary with relatively more eelgrass rearing habitat than most Oregon estuaries. On the other hand, Rogue spring juveniles emerge from beds far upstream (river km 237–251) in a relatively long river system, and migrate through a high flow, steep gradient mainstem and a short, freshwater-dominated estuary. These different environments and life histories might select for divergent timing of smoltification in fish from the two rivers.

The primary objective of this study was to determine whether these two populations differed in the onset of initial salinity tolerance at similar developmental stages as measured by size and (or) age, using the following three physiological parameters of smoltification: percent survival in seawater, final dry/wet weight ratio (water balance) in seawater versus fresh water, and plasma sodium concentration.

Methods

Experimental populations

A group of fish from each population was maintained in 1990 and 1991 for approximately 5 months each year from eyed eggs at the Hatfield Marine Science Center, Newport, Oregon. Fish were compared in a common environment so that observed phenotypic differences could be argued to be of genetic origin. Eyed eggs were obtained from fall chinook salmon adults that had returned to the Trask River Hatchery near Tillamook, Oregon. Trask River eggs were from adults spawned on 31 October 1989 (spawning history unknown) and 30 October 1990 (from a mix of five 1:1 male/female spawning pairs). For the Rogue River spring chinook, eyed eggs were obtained from Oregon Aqua Foods salmon ranch, Springfield, Oregon, in 1990 and Cole River Hatchery, Rogue River, Oregon, in 1991. Rogue River eggs were from adults spawned on 1 November 1989 (F_1 progeny from 3-year-old salmon-ranched, returning adults from Cole River hatchery stock; spawning history unknown) and 24 October 1990 (from a mix of 2 males and 2 females). Rogue River eggs had to be obtained from different sources between experiment years because Oregon Aqua Foods could not provide eggs after 1990 because of financial difficulties; however, Rogue juveniles used in each year were from the same population. Eyed eggs from each population were incubated in Heathman trays supplied with dechlorinated fresh water (8.5–11°C). Eyed eggs were weighed by the hatcheries for the 1990 brood year, but in 1991, I measured eyed egg weight (by displacement) and diameter (with vernier calipers).

As soon as the yolk sacs were absorbed, each population was placed in a separate trough receiving dechlorinated fresh water (ambient water temperature). Fish were fed ad libitum moist fish pellets (Biodiet, Warrenton, Oreg). and times of first feeding were noted. Holding troughs were exposed to ambient photoperiod via skylights. Ambient water temperature was measured daily throughout the holding period. Fork length and wet weight of a subsample of each population was measured every 14–30 days to monitor growth. Measurements were taken from mid-February to April in 1990 and mid-February to late May in 1991. Specific growth rate (*G*) was calculated by the following equation (Laird and Needham 1988):

$$G = \frac{\ln W_t - \ln W_0}{t} \times 100$$

where W_t is weight at time *t*, W_0 is weight at time 0, and *t* is the duration between time *t* and 0.

Survival and water balance experiments

Percent survival and final dry/wet weight (DWW) ratio were used to measure the response of each population to a 96-h exposure to three salinities. The two-way factorial design contained the following variables: (*i*) population:Trask vs. Rogue and (*ii*) salinity:0, 16, and 32 ppt. Each treatment of stock X salinity was replicated three times with 10–15 fish per replicate. Fish were starved for 24 h, anesthetized with MS-222, and individually measured for fork length and wet weight before holding in experimental tanks (static, 16-L, black plastic buckets with aeration) at ambient fresh water temperature and photoperiod. Mortalities were removed and recorded twice daily. Percent survival was expressed as mean percentage of survivors from the three tank replicates per treatment. Experiments were repeated throughout the spring with different individuals from each population. Six experiments were conducted from 9 days until 109 days after first feeding, when water temperature became too warm and fish became too large for experimental chambers. Three experiments were conducted in 1990: 14–18 March, 21–25 March, and 6–10 April; three experiments were conducted in 1991: 24–28 February, 21–25 April, and 19–23 May.

At the end of each experiment, each surviving fish was weighed, dried for 48 h at 60°C, and weighed again to obtain the ratio of dry to wet tissue weight. Because mortalities were only retrieved twice daily, their weight at death could not be accurately measured; therefore, they were not included in calculating the DWW ratio. To determine if each surviving fish had dehydrated over the 96-h period, the final DWW ratio of fish in 32 ppt seawater was compared with the final DWW ratio of fish in the freshwater control. I assumed that when a fish that was acclimated to freshwater, and thus excreting copious urine to regulate internal electrolyte balance, was abruptly put into seawater, it may have either (*i*) adjusted its osmoregulatory system quickly to the surrounding hyperosmotic environment, so the DWW ratio would not have been significantly different in 0 ppt freshwater compared to 32 ppt seawater, or (*ii*) failed to osmoregulate effectively and dehydrated (but still survived) through the loss of water to the surrounding hyperosmotic environment, so wet weight would have decreased relative to dry weight. Therefore, the DWW ratio of fish in 32 ppt seawater would have been statistically greater than the ratio of fish in 0 ppt freshwater.

Plasma sodium concentration experiments

Three 24-h seawater-challenge tests were conducted to coincide with the later 1991 (21–25 April; 19–23 May) survival and water balance experiments (after Blackburn and Clarke 1987). Differences in salinity tolerance were compared by measuring plasma sodium concentrations from both fish populations held in freshwater and 32 ppt. A plasma sodium level ≤ 170 mmol^{-1} was used to indicate full osmoregulatory ability in seawater (Blackburn and Clarke 1987). To obtain enough plasma for analysis, three to four fish were combined per replicate for the first experiment, two to three fish for the second, and two fish for the Rogue population in the third experiment. Fish were starved for 2 days prior to experiments and were held in tanks with flow-through freshwater or seawater at ambient photoperiod and temperature.

After 24 h, fish were quickly killed with an overdose of MS-222, weighed, and measured for fork length. The tail was then cut, and blood from the dorsal aorta was collected with capillary tubes treated with lithium–heparin. Blood was centrifuged for 5 min to separate the plasma from cells and transported within 24 h on ice to the Oregon State University Veterinary Diagnostic Laboratory, Corvallis, Oreg. Plasma samples, sodium standards, and reference sera were analyzed using a Ciba–Corning Na–K analyzer.

Statistics

Survival percentages were arc-sine transformed for analysis (Sokal and Rohlf 1981). Back-transformed data are reported for ease of interpretation. Differences in survival were compared with Student's *t*-tests. DWW ratios were analyzed with a two-way ANCOVA (mean initial wet weight per

Fig. 1. Specific growth rate (% body weight/day) of Trask and Rogue chinook salmon populations in 1990 (*a*) and 1991 (*b*) versus calendar day. Values were calculated from weight measurements used for Fig. 2 and represent the change in growth rate between the contemporary and previous sample dates.

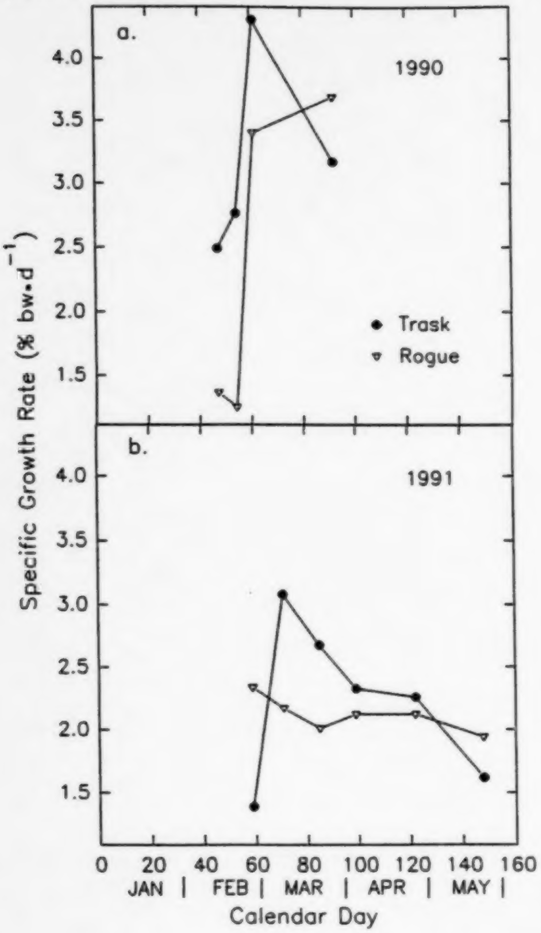
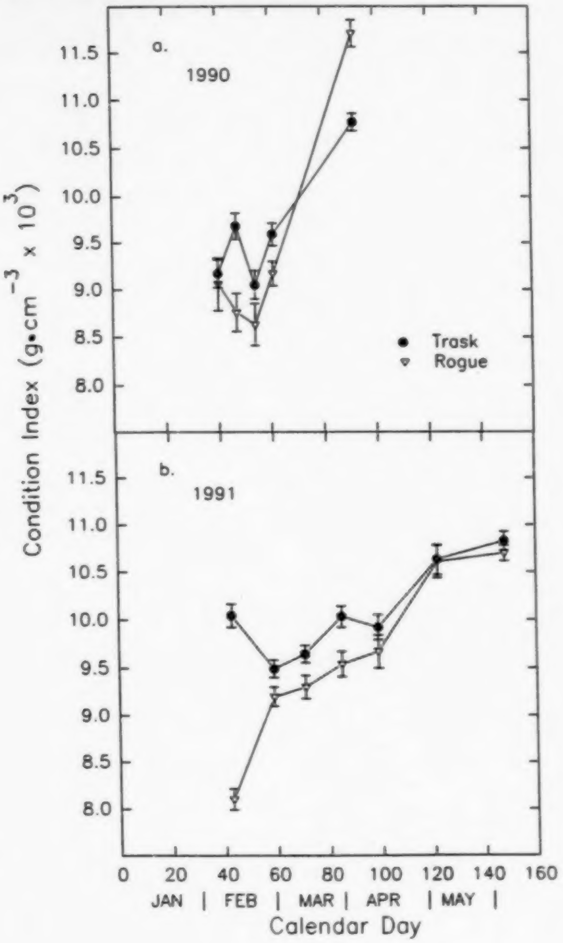


Fig. 2. Condition index (g/cm^3 × 10^3) of randomly chosen individuals from Trask and Rogue experimental populations in 1990 (*a*) and 1991 (*b*) versus calendar day. See Methods for dates of first feeding for each population in each year. Values are means ± SE of 20–30 fish held under identical growing conditions.



population as the covariate) because fish of each population varied in weight at similar ages. I used the Kramer modification of Tukey's multiple range test for statistical comparisons of means (Day and Quinn 1989), which accounts for unequal sample size because of differential mortality among treatments and for experiment-wise error. Data from the 2 years of survival and water balance experiments were pooled for presentation because of the small number of experiments per year ($N = 3$ per year, $N = 6$ total).

Results

Experimental populations

Weights of eyed eggs were similar within each population in both years; however, Trask eggs were significantly ($p \leq 0.0001$) larger and heavier than Rogue eggs in both years. In 1989, weights of eyed eggs were 0.411 and 0.263 g/egg for Trask and Rogue populations, respectively. In 1990, respective weights (±SE) and diameters (±SE) of eyed eggs were 0.461 ± 0.038 g/egg and 9.41 ± 0.15 mm/egg for Trask fish and 0.251 ± 0.01 g/egg and 7.70 ± 0.14 mm/egg for Rogue fish.

The time of first feeding varied between populations and years by up to 15 days. In 1990, Trask and Rogue fish started to feed on 5 February and 1 February, respectively. However, in 1991, 14 days separated the time of first feeding, possibly because of a difference of 1 week in spawning date between populations; Trask and Rogue fish started feeding on 14 February and 30 January, respectively.

Specific growth rates (G) and condition indices (K) differed over time between the populations (Figs. 1 and 2). In

Fig. 3. Initial wet weight (mean ± SE) of fish from Trask and Rogue populations used for each of six (1990 and 1991 combined) survival and water balance experiments (*a*) and percent survival in 32 ppt seawater after 96 h (*b*) for Trask and Rogue populations versus calendar day. See Methods for exact date and year of each experiment. An asterisk represents a significant ($p < 0.05$) difference for a pairwise comparison using Tukey's test.

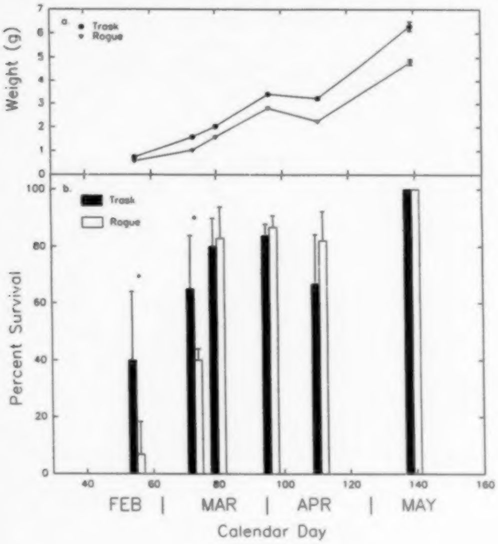
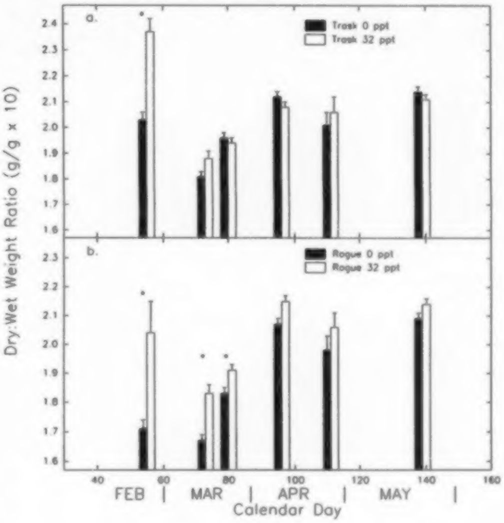


Fig. 4. Final dry/wet weight ratio (DWW; g/g × 10) for Trask (*a*) and Rogue (*b*) fish held in 0 ppt freshwater versus 32 ppt seawater for six experiments (1990 and 1991 combined) versus calendar day. Data represents the mean DDW ± SE of survivors from a 96-h abrupt exposure to either 0 or 32 ppt water. See Methods for exact date and year of each experiment. An asterisk represents a significant ($p < 0.05$) difference for a pairwise comparison using Tukey's test.



1990 and 1991, Trask fish grew more rapidly than Rogue fish until late March in 1990 and early May in 1991, when Rogue fish began to grow faster (Fig. 1). After that, Rogue fish continued to grow more rapidly than Trask fish until the end of measurements for both years. The timing of these changes in growth rate between the two populations corresponds to the same time that the *K* of Rogue fish became greater than that of Trask fish in 1990 (Fig. 2*a*) and to the same time that the *K*'s of both populations converged in 1991 (Fig. 2*b*).

Survival and water balance experiments

Survival of Trask fish in 32 ppt seawater (as early as 10 d post-first feeding) was significantly ($p < 0.05$) greater than Rogue fish until fish reached about 1.5–2 g (5–6 cm fork length (FL); Fig. 3). At weights of about 2–3 g (about 2 months post-first feeding; 6–7 cm FL), >80% of fish of both populations survived in seawater and by the time fish weighted 4–6 g (about 3 months post-first feeding; 7–8 cm FL) survival was 100% in seawater. Fish from both populations survived 100% in 0 and 16 ppt water in all experiments.

If the DWW ratio in 0 ppt did not significantly ($p > 0.05$) differ from the DWW in 32 ppt seawater, I assumed that fish of that population were competently osmoregulating. In late February, about 10 d after first feeding and at weights of <1 g, both populations dehydrated (i.e., DWW in 32 ppt significantly > DWW in 0 ppt) in 32 ppt seawater (Fig. 4). By mid-March the DWW ratio of Trask fish weighing <2 g was similar ($p > 0.05$) in 0 and 32 ppt, and continued to be until experiments ended in late May. The DWW ratios of Rogue fish held at the different experimental salinities, however, did not become similar until early April at weights >2 g.

Percent survival indicated a different minimum size and timing of the onset of salinity tolerance than the DWW ratios. For example, fish from both populations showed no differences in percent survival in 32 ppt seawater by the late March experiment; however, Rogue fish were still dehydrating in 32 ppt seawater relative to 0 ppt freshwater at this time. Rogue fish showed no difference in DWW ratio between 0 and 32 ppt water until the next experiment in early April, indicating increased salinity tolerance. Although experiments were not conducted at the same time each year, thus enabling direct comparison between years, survival, and water balance data did not vary within populations between similar-dated experiments in 1990 and 1991.

Plasma sodium concentration experiments

Plasma sodium concentrations indicated that both populations were not osmoregulating competently at the time of the first seawater challenge on 24 April (Table 2), although most fish were surviving and not dehydrating. By the next challenge on 21 May, the performance of both populations had improved, as indicated by their regulation of plasma sodium concentration near or below 170 mmol/L (Blackburn and Clarke 1987). However, perhaps because of

Table 2. Plasma sodium concentrations (mmol/L) for Trask and Rogue juvenile chinook salmon held in either 0 or 32 ppt water for 24 h during spring 1991.

	Size (mean ± SD, g)			Treatment (mean ± SD, mmol/L)			
Date	Trask	Rogue	Temperature (°C)	Trask, 0 ppt	Rogue, 0 ppt	Trask, 32 ppt	Rogue, 32 ppt
24 April	3.33±0.75	2.46±0.77	10.9–12.2	169.2±17	155.3±7.5	192.5±14.5	193.9±14.9
21 May	6.92±0.98	5.09±1.03	12.6–13.4	169.3±4.0	159.6±4.1	165.9±5.4	171.9±7.6
12 June	8.96±1.59	6.59±1.53	13.6–15.2	166.2±3.8	160.8±8.6	181.3±9.0	182.3±9.1

Note: Duplicates of race × salinity treatments were combined. "Temperature" is the range of temperatures in the fresh water holding tanks for 2 weeks prior to each experiment.

increasing fresh water temperature in the population holding tanks, the performance of both populations decreased by the 12 June challenge, although plasma sodium concentrations were not as high as on the 24 April challenge.

Discussion

When held under identical conditions, fall chinook salmon juveniles from the Trask River survived better and did not dehydrate in 32 ppt seawater relative to 0 ppt freshwater at a smaller size and younger age than did Rogue River spring chinook juveniles. Other researchers have similarly shown that chinook with contrasting life histories may also differ in their development of hypoosmoregulatory capabilities. Taylor (1990*c*) found that the juvenile life history of adult chinook within a single population was moderately indicative of the salinity tolerance of their offspring. Juveniles from parents with more juvenile freshwater circuli, which is suggestive of a longer freshwater residence time as juveniles, showed greater percentage weight loss in 20 ppt seawater and lower mean survival in 30 ppt seawater in their first 3 months after emergence. However, Taylor (1990*c*) cautioned that after adjustment for different sizes at emergence, among-family differences in physiological performance within a population still persisted. This was also a concern in my study because I could not control the pairing of parents at the time of spawning, as well as the size of eyed eggs and resulting alevins, which in turn can influence early feeding and growth, and perhaps salinity tolerance. In addition, Carl and Healey (1984) found that progeny survival of adult chinook from different spawning reaches in the Nanaimo River system, British Columbia, differed significantly when exposed to seawater for 7 days. Juveniles from lower river spawning sites showed significantly greater survival (97.5%) in seawater than offspring from mid-river beds (86.9%).

Clarke et al. (1992) offered direct genetic evidence for a difference in smolting phenotype between ocean-type and stream-type chinook. Progeny of ocean-type parents became ocean-type smolts, and progeny of reciprocal crosses between ocean- and stream-type parents became ocean-type smolts, indicating that the ocean-type smolting pattern is dominant and not under maternal control. The smolting pattern of backcrosses of F_1 hybrids between ocean- and stream-type populations depended on parental history. When exposed to the same photoperiod history as their parents, backcrosses to ocean-type parents produced ocean-type smolts, indicating Mendelian inheritance of a single major gene for this aspect of chinook smoltification (W.C. Clarke et al., unpublished data (Abstr.), 1991 Annual Meeting American Society of Zoologists). Given my results and those of Taylor (1990*c*) and Clarke et al. (1992), my data are consistent with a genetic difference between Trask and Rogue river chinook salmon with respect to smolting physiology.

However, possible maternal effects in the Trask river population, i.e., that it exhibits significantly larger eggs at a similar age compared with the Rogue population, may confound a strictly genetic interpretation of the observed differences in the onset of salinity tolerance. The significant difference in egg size between Trask and Rogue populations may be related to their contrasting life histories, especially their respective estuarine versus freshwater rearing habitats. Taylor (1990*c*) argued that if a genetic component to female size at maturity and therefore egg site exists, then the larger size at emergence (and first feeding) of estuarine-rearing juveniles may be an adaptation to rearing in a saline environment, bestowing early enhanced salinity tolerance. Given the positive relationship between size and salinity tolerance of fish used in this study, the larger eggs and size at emergence of Trask River fall chinook juveniles are consistent with Taylor's (1990*c*) hypothesis.

Changes in specific growth rate (*G*) and condition index (*K*) over time (Figs. 1 and 2) for all experimental populations also imply a preparation by the Trask population for earlier rearing in a saline environment; i.e., by mid-spring, the *G* of Trask fish had slowed and they became longer and thinner relative to Rogue fish. Similarly, Carl and Healey (1984) found that chinook populations in the Nanaimo River, British Columbia, that reared in high salinity estuarine habitats had slimmer bodies, smaller heads, and shorter fins than two other populations that reared in either the freshwater part of the river for 2 months or in fresh water for 1 year. When comparing the *G* of ocean-type versus stream-type juveniles over 12 months, Taylor (1990*b*) found that ocean-type chinook grew faster. Assuming that Trask fish behave more like ocean-type chinook, given their juvenile life history and earlier onset of salinity tolerance, the change in *G* over time for the Trask experimental population agrees with the findings of Taylor (1990*b*). At the start of the experiments, in both years, Trask fish grew faster than Rogue fish; however, as the season progressed, the *G* of Trask fish slowed while the *G* of Rogue fish increased.

The suite of evidence for the Trask population (larger initial egg size and early rapid growth, decrease in condition index, and early onset of salinity tolerance at a small size) suggests a genetically programmed schedule of changes in physiology that probably enhances their ability to survive in saline water at a smaller size and younger age than Rogue River juveniles. When decoupling size and age in comparing *G*'s between the populations, this idea remains consistent. For example, in 1991, the *G* for Rogue fish between sizes 0.83 ± 0.19 and 1.10 ± 0.36 g was 2.01, whereas, the *G* for Trask fish between similar sizes (0.85 ± 0.10 and 1.3 ± 0.24 g), but at an earlier age, was 3.08. Also, in the next-similar size range for 1991, the *G* for Rogue fish between 1.10 ± 0.36 and 1.48 ± 0.55 g was 2.12, and the *G* for Trask fish between 1.23 ± 0.34 and 1.79 ± 0.34 g was 2.68.

Plasma sodium concentrations of fish from both populations indicated that they were not osmoregulating competently in 32 ppt seawater at sizes of 2.5 (Rogue) to 3.3 g (Trask) and at the age of about 3 months post-first feeding. However, by about 4 months post-first feeding and sizes of 5 (Rogue) to 7 g (Trask), fish from both populations were osmoregulating competently. These results agree with the development of seawater tolerance by fall chinook juveniles from the Big Qualicum hatchery, British Columbia, studied by Clarke and Shelbourn (1985). They found that juveniles weighing ≥1.5 g could survive and grow in seawater, but the optimum timing and conditions for regulation of plasma sodium concentrations were found when 5.6 g fish were transferred from 13.8°C fresh water to 10.2°C on 18 May. Even though fish in the present study had high concentrations of sodium in their plasma at the time of the first seawater challenge (24 April), they were surviving in high proportions and not dehydrating. Perhaps the fish in my experiments showed an increased resistance to salinity, despite high plasma sodium concentrations, because, as Weisbart (1968) suggested, the organs of chinook salmon can perform normally in a wider range of osmotic environments than those of coho or sockeye salmon.

In summary, Trask River juvenile chinook salmon were more favorably adapted physiologically to enter an estuarine environment at an earlier age and smaller size than Rogue fish as measured by physiological parameters. This may be an adaptation to the well-developed estuarine habitat of the Trask River. By contrast, Rogue River chinook appear to be adapted to take advantage of a freshwater environment. These populations differed in their timing and minimum size at which development of salinity tolerance occurred, thus lending support to McCormick and Saunders' (1987) idea that adaptive mechanisms play a role in the evolution of saltwater entry. The minimum size and (or) age of salinity tolerance, however, depended on which indicator was chosen for study. For example, an acute measure of salinity tolerance such as percent survival indicated that both populations tolerated seawater at 6 weeks post-first feeding (>80%, 1.5–2 g fish). However, chronic measures of salinity tolerance such as final dry/wet weight ratio indicated that Rogue fish could not effectively osmoregulate until 8 weeks post-first feeding (>2 g), but Trask fish attained osmoregulatory ability at 50 days post-first feeding (<2 g).

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Fish production in freshwaters: Are rivers more productive than lakes?

R.G. Randall, J.R.M. Kelso, and C.K. Minns

Abstract: Community fish production data were summarized from the literature to test the hypothesis that production is higher in rivers than in lakes. Average community production at 55 river sites was three times greater (273 $kg \cdot ha^{-1} \cdot year^{-1}$) than at 22 lakes (82 $kg \cdot ha^{-1} \cdot year^{-1}$). Higher production ($P$) in rivers resulted from much higher densities of fish (14 times) and greater biomass (B) (about 2 times). Average fish weight and P/B ratios were inversely correlated. Average fish weight was 7 times less, and P/B ratios were 1.5 times higher (after correction for fish size), in rivers than in lakes. Thus, rivers not only had higher average biomasses of fish but also the turnover rate of the biomass was greater. Fish production was positively correlated with phosphorus in both lakes and rivers. Information on community fish production supported the hypothesis that the productivity of river habitat is, on average, higher than the productivity of lake habitat. The productive capacity of freshwater habitats can be predicted using a multiple regression model developed by Boudreau and Dickie (Can. J. Fish. Aquat. Sci. **46**: 614. 1989), where fish production is calculated from average fish biomass and body mass.

Résumé : Nous avons compilé des données sur la production des communautés de poissons présentées dans la littérature pour vérifier l'hypothèse selon laquelle la production serait plus élevée dans les cours d'eau que dans les lacs. La production annuelle moyenne des communautés de 55 sites lotiques était trois fois plus élevée (274 $kg \cdot ha^{-1} \cdot a^{-1}$) que celle de 22 sites lacustres (82 $kg \cdot ha^{-1} \cdot a^{-1}$). La production ($P$) plus élevée observée dans les cours d'eau provenait du fait que la densité des poissons y était beaucoup plus élevée (14 fois) et la biomasse (B) plus grande (à peu près 2 fois). Le poids moyen des poissons et les rapports P/B étaient inversement corrélés. Le poids moyen des poissons était 7 fois plus faible, et les rapports P/B 1,5 fois plus élevés (après correction en fonction de la taille des poissons) dans les cours d'eau que dans les lacs. Ainsi, non seulement les cours d'eau présentaient-ils en moyenne des biomasses de poissons supérieures, mais le taux de renouvellement de la biomasse y était aussi plus élevé. La production de poissons était positivement corrélée au phosphore tant dans les lacs que dans les cours d'eau. Les données sur la production des communautés de poissons ont confirmé que la productivité de l'habitat lotique est en moyenne plus élevée que celle de l'habitat lacustre. On peut prédire la capacité de production des habitats dulcicoles à l'aide d'un modèle de régression multiple mis au point par Boudreau et Dickie (Can. J. Fish. Aquat. Sci. **46**: 641. 1989), selon lequel on calcule la production de poissons à partir de la biomasse moyenne et de la masse corporelle moyenne des poissons.

[Traduit par la Rédaction]

Introduction

Are freshwater habitats in rivers more productive than in lakes? In a review of the differences in biotic and abiotic structure between rivers and lakes, Ryder and Pesendorfer (1989) stated that rivers are "typically more productive than their standing-water counterparts." Energy sources and resulting trophodynamics of biological communities in rivers and lakes are very different. Biotic communities of rivers, particularly in headwater reaches, are often heterotrophic and depend on allochthonous detritus as sources of energy. Lakes on the other hand are autotrophic, and much of the primary production that fuels the energetics of the biotic community occurs within the lake system itself. River ecosystems are therefore more closely associated with their terrestrial environment than are lakes, in both a spatial and energetic sense. Because of the availability of the allochthonous food base which is funnelled into the stream or river channel from the surrounding drainage basin, animal production may be greater in rivers than in lakes.

The hypothesis that fish population per unit area is greater in rivers than lakes is addressed in this paper. Estimates of community fish production from the literature are used to test this hypothesis. In addition to fish

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production, we compare the impact of fish density, average fish weight, biomass, production to biomass ratios (P/B), and species richness on production. We assume that fish production in both habitats is limited by nutrients, and thus is "bottom-up" rather than "top-down" regulated (see discussion of the concepts of bottom-up and top-down in Northcote 1979). Fish production or biomass has been shown to be positively correlated to production at lower trophic levels, or to phosphorus concentration which is an indicator of water productivity (Peters 1986), in both lakes (Downing et al. 1990) and rivers (Hoyer and Canfield 1991). Hence, for this study, we assume a direct link between habitat productivity and community fish production. Enough estimates of community fish production are now available from the literature to allow us to begin making generalizations about differences in the productive capacity of fluvial and lentic habitats.

Boudreau and Dickie (1989) developed a multiple regression equation that predicts fish production from average biomass and body mass of the fish in the age group or population being studied. A second objective of this study was to determine if Boudreau and Dickie's model could be used to predict fish community production in both lake and river habitats.

Methods

Data on fish production or biomass of entire fish taxocenes were available for 31 lakes and 62 river sites distributed throughout the world. For lakes, we began with the synthesis of data from 20 lakes which was summarized by Downing et al. (1990). To this, we added data for an additional 11 lakes from data found in the literature but not used by Downing et al. (1990). Average fish production and biomass from 19 small lakes in Ontario was treated as one data point, as the lakes were similar in size and physical nature (Kelso and Johnson 1991), and thus were considered as replicates (J.R.M. Kelso, personal observation). For rivers, we utilized the data summarized by Chapman (1978), Welcomme (1985), and Mann and Penczak (1986). Some of the river estimates were from different tributaries of the same river (e.g., Utrata River in Poland; Penczak 1981); we included these as separate estimates if the number of fish species or their densities were different among tributaries. Average fish biomass for several rivers in six states of United States and from Ontario, Canada (Hoyer and Canfield 1991) was also used. The latter data included total phosphorus concentrations in rivers and allowed us to compare phosphorus and fish relationships in rivers and lakes.

For both rivers and lakes, we avoided estimates of production of single species or families of fish if these represented only a portion of the total fish community (and hence biomass). Although considerable data on single species exist, particularly for salmonids in coldwater streams (Mann and Penczak 1986), we were interested in total community estimates only, as these were more likely to reflect carrying capacity. Estimates of production of single fish species were included only if the species occurred allopatrically (e.g., Char Lake).

When available, the following data were included in our analysis: mean biomass (B, kg·ha^{-1}, annual production (P, kg·ha^{-1}·year^{-1}), species richness (SR, total number of species inhabiting the area), and density (D, number·ha^{-1}). Density information was usually for the time of sampling. When average biomass data were not available, standing biomass at the time of sampling was used. Population abundance was usually estimated by mark–recapture methods or, particularly in rivers, by removal methods. For most studies, production was estimated as the product of specific growth rate (G) and mean biomass (B), using algebraic or graphic techniques as outlined by Chapman (1978). Data for lakes and rivers are summarized in Appendices 1 and 2, respectively.

From the published estimates of mean biomass and annual production, we calculated the production/biomass ratio (P/B) for the entire fish community. The production to biomass ratio was used to indicate the growth or turnover rate of the community biomass. In addition, mean weights (W, g) of fish in the communities were calculated by dividing mean biomass (g·ha^{-1}) by fish density (D, number·ha^{-1}).

Differences in fish density, biomass, and production between lakes and rivers were tested using nonparametric procedures (Mann–Whitney U-test; Zar 1974). Correlations between production and biomass, density, and species richness were tested using standard regression analysis. Data were log transformed (base 10) for all regression analyses, to stabilize variances and to linearize the relationships between them. When significant regressions were found, differences in slopes and elevations between lake and river data were tested using a dummy variable, where lakes were assigned 0 and rivers 1 (Neter et al. 1983; Wilkinson 1990). This is analogous to an analysis of covariance. Results of some of the slope tests should be viewed with caution, however, because for some covariables (W and D) there was little overlap between the lake and river data sets. When significant differences in elevation (but not slopes) were found, statistics were given for the separate lake and river regressions, and for the regression model including the dummy variable for the pooled data (common slope model).

Results

Fish production was on average higher in rivers than in lakes. Production in rivers ranged between 26 and 2800 kg·ha^{-1}·year^{-1} (average 273 kg·ha^{-1}·year^{-1}), while production in lakes ranged between 2 and 398 kg·ha^{-1}·year^{-1} (average 82 kg·ha^{-1}·year^{-1}) (Table 1). In addition, related variables, namely species richness, fish density, and biomass, were all on average higher in rivers than in lakes (Fig. 1 and Table 1). Differences were statistically significant (Mann–Whitney U-test, $p < 0.05$; Table 1) for all community measures except species richness. The magnitude of the difference in production variables between rivers and lakes was considerable: average density was 14 times, biomass was 2 times, and average production was 3 times greater in rivers than in lakes. However, average fish weight was 7 times less in rivers than in lakes.

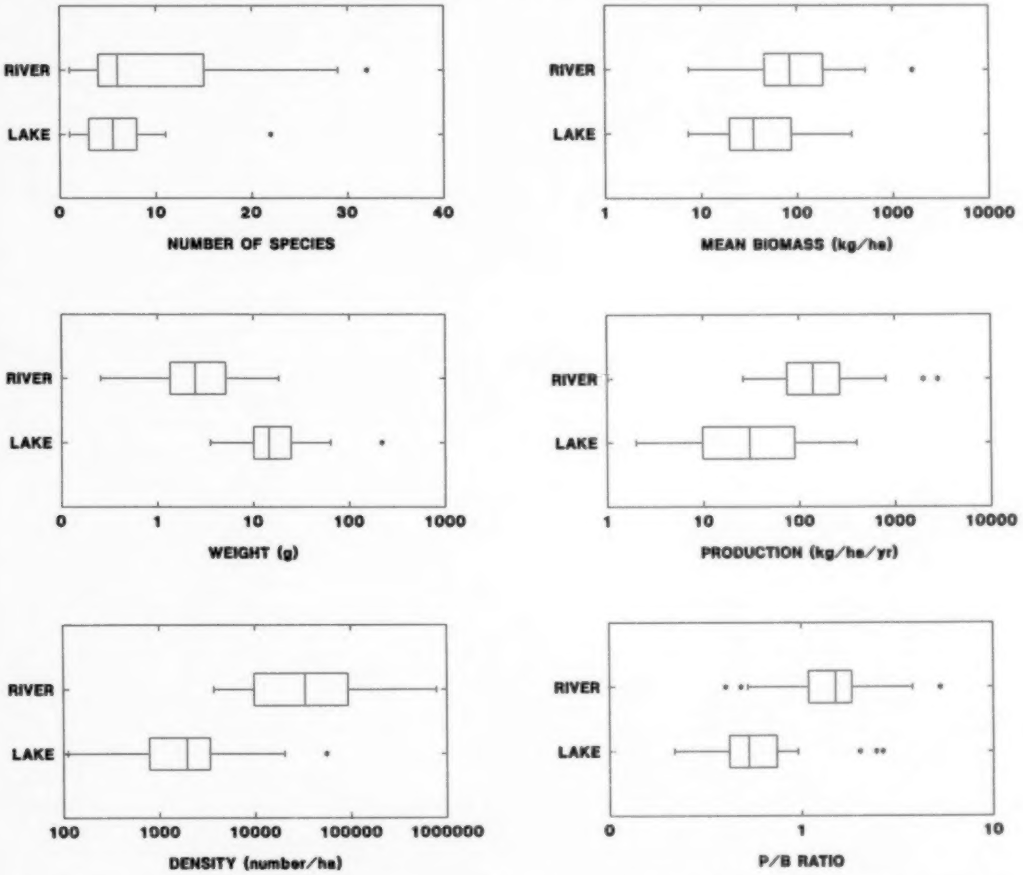
Fish weight was negatively correlated with fish density in both lakes and rivers (Fig. 2). Regression slopes for the river and lake data were not significantly different ($p > 0.05$), but intercepts were different ($p < 0.003$), indicating that density was 3.1 times higher (i.e., $10^{0.49}$, Table 2) for rivers than

Table 1. Comparison of production and related variables between lakes and rivers.

Variable	Sample size		Mean value		U	p	Ratio of rivers/lakes
	Lakes	Rivers	Lakes	Rivers			
Production (kg·ha^{-1}·year^{-1})	22	55	81.8	273.0	275	0.000	3.3
Species (no.)	20	47	6.3	9.4	387	0.254	1.5
Density (no.·ha^{-1})	19	42	5577	75 665	47	0.000	13.6
Weight (g)	19	42	28.9	4.1	737	0.000	0.1
Biomass (kg·ha^{-1})	31	58	83.8	146.1	570	0.005	1.7
P/B ratio	22	51	0.78	1.63	185	0.000	2.1

Note: Statistical differences were tested using the non-parametric Mann–Whitney U-test. Data sources are given in Appendices 1 and 2.

Fig. 1. Box plots to compare fish species richness, mean weight, density, biomass (B), production (P), and P/B ratio in lakes and rivers. The left and right side of the box show the 25th and 75th centiles of the data, respectively (interquartile range). The vertical line through the box indicates the median, the "whiskers" represent 1.5 times the interquartile range, and the asterisks indicate points which are greater than 1.5 times the interquartile range (Systat for Windows 1992). Sample sizes for each comparison are given in Table 1.



lakes for any given mean fish size. Slopes of both regressions were not significantly different from −1.0 (Table 2).

Differences in fish weight between lakes and rivers also affected the P/B ratios. Mean P/B was 1.7 times higher in rivers (1.63) than in lakes (0.78). P/B ratios were significantly and negatively correlated with mean weight in both rivers and lakes (Fig. 3). Slopes of the regressions of P/B with W were not significantly different between rivers and

Table 2. Regression equations relating fish density and fish weight.

Habitat	Model	R^2	n	p
Lakes	$\log D = 4.48(0.37) - 1.01(0.29) \log W$	0.38	19	0.003
Rivers	$\log D = 4.90(0.09) - 0.94(0.15) \log W$	0.50	42	<0.001
Pooled	$\log D = 4.41(0.19) - 0.96(0.13) \log W + 0.49(0.16)\, ID$	0.74	61	<0.001

Note: D is fish density (no.·ha^{-1}) and W is the mean weight (g) of the fish in the community. The regression for the pooled data included a dummy variable (ID) for lakes (0) and rivers (1). The coefficient of determination is adjusted for the number of parameters in the model. Standard errors for the regression coefficients are given in parenthesis.

Fig. 2. Correlation between mean fish weight (g) and fish density (no./ha) of fish communities in lakes (triangles and dashed line) and rivers (circles and solid line). Regression coefficients are given in Table 2.

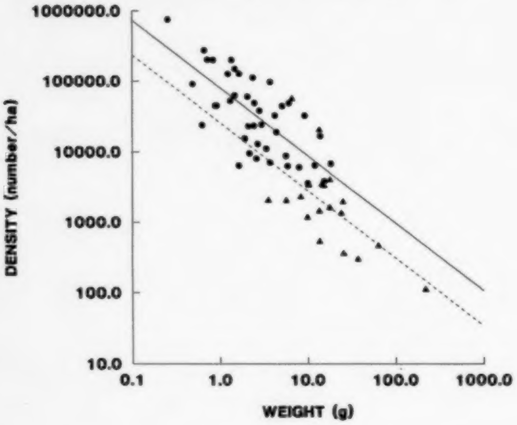
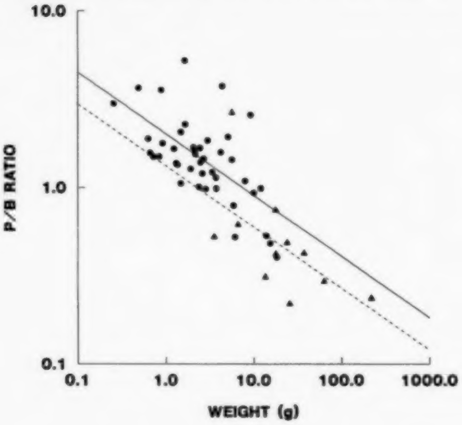


Fig. 3. Correlation between mean fish weight (g) and P/B ratios of fish communities in lakes (triangles) and rivers (circles). Regression coefficients are given in Table 3.



lakes ($p > 0.05$; Table 3). However, intercept coefficients of this regression were significant ($p = 0.044$), indicating that P/B ratios were 1.5 times greater in rivers than lakes ($10^{0.18}$, Table 3) after adjustment for fish size. The sample size for lakes was small ($n = 11$). On average, however, fish biomass was not only higher in rivers, but also the growth of biomass was significantly greater in rivers than in lakes.

Production was not correlated with species richness in either lakes or rivers (Fig. 4*a*). As expected, however, production was significantly correlated with both fish density and biomass (Figs. 4*b* and 4*c*). For fish densities, the slopes and intercepts in the regression between density and production were not significantly different ($p > 0.05$), suggesting that for any given density, production was similar in lakes and rivers. For fish biomass, slopes for the regression between biomass and production in lakes and rivers were not different ($p = 0.12$), but the intercepts were significantly different ($p < 0.001$). For any given biomass, fish production was 2.4 times higher in rivers than lakes ($10^{0.38}$, Table 4). The different intercepts reflected the differences in P/B ratios noted earlier. Coefficients of determination for the regressions that related production to density or biomass were all greater than 70% (Table 4).

Boudreau and Dickie's equation for predicting production was $\log_{10} P = \log_{10} a + b\log_{10} W + c\log_{10} B$, where P is production rate, W is body mass, and B is average biomass. This multiple regression equation was significant for both the rivers and lakes ($p < 0.001$; Table 5). The coefficient of determination for the pooled data from lakes and rivers was 0.89. Biomass was the dominant factor affecting production, but inclusion of the W component increased the coefficient of determination by 9% for lakes, 12% for rivers, and 19% for the pooled data. Again, the sample size for lakes was small ($n = 11$). Intercepts (a) of the lake and river data were significantly different ($p = 0.016$).

Many investigators did not provide information on phosphorus levels at their study sites. However, total phosphorus concentrations were available for 12 lakes (Downing et al. 1990) and 7 river data points (Hoyer and Canfield 1991). For the combined lake and river data, fish biomass and phosphorus concentrations were significantly correlated ($p < 0.01$; Fig. 5). Fish biomass was similar for any given phosphorus concentrations in both types of habitat. The Lake Nakuru data was left out of the regression because preliminary analysis indicated that it was a significant outlier (Studentized residual = -3.48). Lake Nakuru is a hypereutrophic lake and phosphorus concentrations were several-fold greater than for any of the other lakes in the data set (Appendix 1). Excluding the Lake Nakuru data point, phosphorus levels were significantly higher in rivers than lakes (Mann–Whitney U-test, $p = 0.051$).

Table 3. Regression equations relating specific production (*P*/*B*) to fish weight.

Habitat	Model	R^2	n	p
Lakes	$\log P/B = 0.15(0.22) - 0.37(0.16) \log W$	0.32	11	0.041
Rivers	$\log P/B = 0.30(0.04) - 0.34(0.07) \log W$	0.37	42	<0.001
Pooled	$\log P/B = 0.12(0.10) - 0.35(0.06) \log W + 0.18(0.09)\,ID$	0.61	53	<0.001

Note: *P*/*B* is the ratio of production to biomass (both in kg·ha^{-1}) and *W* is the mean weight (g) of the fish in the community. Standard errors for regression are given in parenthesis.

Table 4. Regression equations relating production to fish density or biomass.

Dependent variable and habitat	Model	R^2	n	p
Density				
Lakes	$\log P = -0.98(0.41) + 0.67(0.13) \log D$	0.74	11	<0.001
Rivers	$\log P = -0.57(0.31) + 0.59(0.07) \log D$	0.64	42	<0.001
Pooled	$\log P = -0.86(0.19) + 0.65(0.05) \log D$	0.80	53	<0.001
Biomass				
Lakes	$\log P = -0.37(0.21) + 1.09(0.12) \log B$	0.80	22	<0.001
Rivers	$\log P = 0.38(0.15) + 0.89(0.07) \log B$	0.74	51	<0.001
Pooled	$\log P = -0.16(0.12) + 0.97(0.06) \log B + 0.38(0.07)\,ID$	0.82	73	<0.001

Note: *P* is production (kg·ha^{-1}·year^{-1}), *B* is biomass (kg·ha^{-1}), *D* is fish density (no.·ha^{-1}), and *W* is the mean weight (g). The regression for the pooled data for biomass includes a dummy variable (*ID*) for lakes (0) and rivers (1). Standard errors for regression coefficients are given in parenthesis.

Table 5. Regression equations relating production to body size and biomass.

Habitat	Model	R^2	n	p
Lakes	$\log P = 0.30(0.33) - 0.38(0.16) \log W + 0.91(0.14) \log B$	0.83	11	<0.001
Rivers	$\log P = 0.51(0.15) - 0.33(0.07) \log W + 0.89(0.07) \log B$	0.80	42	<0.001
Pooled	$\log P = 0.28(0.14) - 0.35(0.06) \log W + 0.90(0.06) \log B + 0.22(0.09)\,ID$	0.89	53	<0.001

Note: *P* is production (kg·ha^{-1}·year^{-1}), *W* is the mean weight (g), and *B* is biomass (kg·ha^{-1}). For the pooled data, *ID* is a dummy variable for lakes (0) and rivers (1). Standard errors for regression coefficients are given in parenthesis.

Discussion

Fish biomass and production data from 62 river sites and 31 lakes summarized from the literature for this study indicated that productivity in rivers is generally higher than in lakes. Higher production in rivers was related to significantly higher fish densities and biomass, and to the smaller weight of fish than in lakes. Our synthesis of the literature data on fish biomass and production support Ryder and Pesendorfer's (1989) contention that the productivity of fluvial habitats should be higher than standing waters.

More energy would be required per unit area in rivers than lakes to support the higher production rates. In addition to the energy costs of growth and production, the energy required for respiration to maintain the biomass would also be higher in rivers than in lakes because of the differences in mean fish size. Mass specific metabolism decreases with fish size (Peters 1983), and thus a given energy input would support less biomass of smaller fish than larger fish. Energy costs of respiration would be higher for the average river community of fish than the average lake community.

The data on phosphorus concentrations, although limited, were consistent with the hypothesis that river productivity was higher, since phosphorus concentrations and fish production were both higher in rivers than in lakes. Phosphorus is routinely used as an indicator of water fertility in lakes because of its direct affect on autotrophic primary productivity (e.g., Peters 1986). Recent data from running water habitats (Hoyer and Canfield 1991; Fig. 5) indicate that phosphorus (or other nutrient elements) may become an important indicator of productivity of rivers as well. The role of phosphorus in lakes and rivers may be different, however. In rivers, phosphorus may have a limiting role on the processing of allochthonous detritus, as well as on autotrophic primary productivity (Elwood et al. 1981; Hoyer and Canfield 1991).

The productive capacity of the habitat determined the amount of fish biomass that was supported, but other factors determined how that biomass was distributed among species and individuals within lake and river ecosystems. Fish species richness was on average higher, although not significantly, at the river sites than at the lake sites. Eadie et al. (1986), while studying species–area relationships of

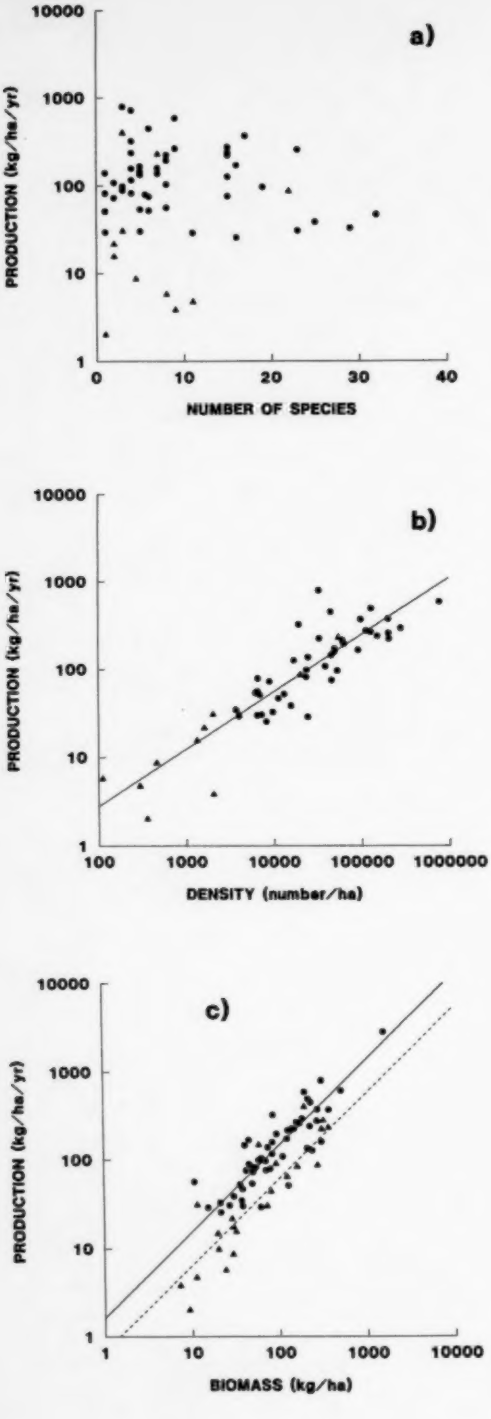
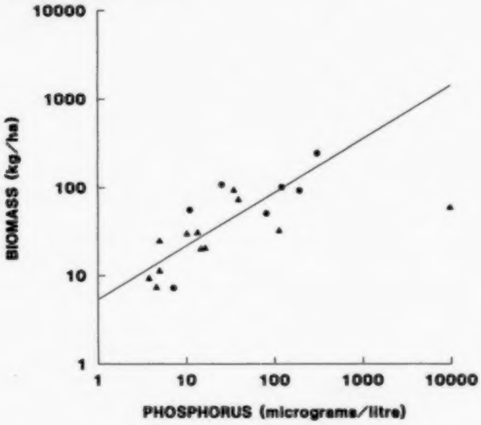


Fig. 4. (*a*) Scattergram comparing species richness (number of species) and community fish production in lakes (triangles) and rivers (circles). (*b*) Correlation between fish density and community fish production in lakes and rivers. (*c*) Correlation between biomass and fish production in lakes (dashed regression line) and rivers (solid line). Coefficients of determination for all regressions are given in Table 4.

Fig. 5. Correlation between average seasonal phosphorus concentrations in the water and fish biomass in lakes (triangles) and rivers (circles). The regression line excludes the data point for Lake Nakuru (extreme right) which was a significant outlier.



fish faunas, found that rivers supported more species of fish per unit area than lakes in Ontario. They attributed this difference to habitat diversity, arguing that rivers provide a greater diversity than lakes. Species richness did not contribute to the observed differences in fish production within the two habitats, however. The relationship between species richness and community production is not well understood for fish populations. Chapman (1978), citing salmonid data from western United States, noted that total production of fish species living sympatrically was higher than species living allopatrically. In conformity with this, the biomass of fish in the 19 Ontario lakes (Kelso and Johnson 1991) was significantly correlated with species richness. Similarly, Downing and Plante (1993) found that fish populations in lakes with greater numbers of fish species had higher rates of production than lakes with fewer species. However, total fish community production was marginally lower than fish population production for a given biomass. Downing and Plante (1993) speculated that intraspecific competition lowered the rate of production of a given biomass if this fish biomass was partitioned into many species. Our data set did not indicate any clear relationship between production and the number of species present in the lake or river survey areas. Similarly, Watson and Balon (1984) did not find any correlation between the number of fish species and production, despite significant differences in community structure among and within several tropical and temperate rivers.

Physical habitat characteristics also affected fish size and fish density. Small size provides a morphological advantage for fish that inhabit the flowing habitats of rivers. Small rheophilic species can seek refuge in the interstices of cobble substrates or other instream structures, can readily exploit allochthonous food sources, and can mature and complete their life cycle at a young age (Welcomme 1985). Schlosser (1990) found that upstream–downstream gradients in environmental characteristics in the Illinois River resulted in different life-history attributes of the fish communities within the river system. Upstream communities of fishes had a shorter life span, smaller body size, and were more abundant than downstream communities. Our results that show higher densities and lower body size for river versus lake species are thus consistent with Schlosser's observations, and extend the links between habitat and fish size/life history characteristics into the lake habitats proper.

Sites with the highest densities of fish, particularly in rivers, were associated with fish communities with lower average fish sizes. Low fish weights at high densities would explain why densities were 14-fold greater, but biomass was only twofold greater, in rivers than in lakes. Correlations between animal body size and density are well established in the literature (Peters 1983; Peters and Wassenberg 1983; Begon 1986; Bohlin et al. 1994). Our slope for fish communities in rivers and lakes was -0.96 ($n = 61$, $SE = 0.13$) and was not significantly different from the theoretical value of -0.9 predicted by the "energetic equivalence" hypothesis discussed by Bohlin et al. (1994). The energetic equivalence hypothesis predicts that if an animal requires a certain area to maintain its metabolic requirements (i.e., animal density is food limiting), then there will be a negative linear relation between log density and log size (weight), with a slope of $-b$. For fishes, the slope is expected to be about -0.9 (Bohlin et al. 1994). The observed relationship between density and fish size in the lake and river habitats suggests that a biomass carrying capacity was reached or nearly reached at a number of the sites. That is, we would not expect to see a significant relationship between fish density and fish size if community fish production was less than carrying capacity. The significantly different intercepts between lakes and rivers (Table 2) are consistent with the different productivities of the two environments. Our contention that fish production, when summed for all species in the community, would approximate productive capacity is supported. Further research needs to be done to better understand the relationship between abundance and animal size in ecosystems (Lawton 1990), particularly since these variables constitute biomass and influence production. As it stands, the density/fish size relationship (Fig. 2) can be used to predict fish abundance from average fish size. Estimates will be imprecise, however, because of the wide variance associated with the regression. Fish size was a weak predictor of fish abundance for both lakes and rivers.

The slope of the *P*/*B* versus weight regression of -0.35 ($n = 53$, $SE = 0.06$) is similar to coefficients found by Dickie (1972), and the "ecological scaling factor" of Dickie et al. (1987*a*, 1987*b*) and Boudreau and Dickie (1989). The different intercept between the lake and river data is again consistent with the different productivity hypothesis and suggest that for any given mean weight, biomass turnover or growth is greater for rivers than lakes. This difference may be related to the physical environment. Nixon (1988) argued that certain marine ecosystems are more productive than lakes because of the influence of the mechanical energy from tides. Mechanical energy, or the kinetic energy of moving water, may influence productivity in rivers as well. An obvious example is organic drift in rivers which offers a continuous supply of food organisms to fish, while in lakes, fish must expend energy while foraging for food (Ryder and Pesendorfer 1989). *P*/*B* ratios are often used in field research to estimate production from biomass data when growth and mortality rates are not known (Banse and Mosher 1980). This study indicates that production can be estimated from biomass data from both rivers and lakes, but *P*/*B* ratios must first be adjusted for habitat (rivers versus lakes) as well as for fish size.

Differences in the intercept *a* of the Boudreau and Dickie (1989) equation ($\log_{10} P = \log_{10} a + b\log_{10} W + c\log_{10} B$) provided the best overall measure of the differences in production between lakes and rivers in this paper. Partial slopes with body weight (*b*) and biomass (*c*) for lakes or rivers, reported in Table 5, were not significantly different from the partial slopes reported by Boudreau and Dickie (1989) (-0.32 and 1.01, respectively). The Boudreau and Dickie multiple regression incorporates the differences in both *P*/*B* ratios and densities, after correction for fish size, between lakes and rivers, which were discussed previously. The significant different intercept (*a*) between lakes and rivers indicated that production was 1.7 times higher ($10^{0.22}$, Table 5) in rivers than lakes, after adjustment for fish size and biomass. The intercept of the Boudreau and Dickie equation is apparently dependent on habitat productivity. It will also be influenced by how habitat area is measured (Boudreau and Dickie 1989), by the units of *W* and *B*, and possibly on whether the production estimates are at the cohort, population, or community level.

The production data summarized from the literature were not originally collected to address whether fish production in lakes and rivers are different. There are obviously limitations to the data set. For example, although most of the lake and river data were from temperate regions, a higher proportion of the river sites were from tropical or subtropical areas than the lake sites. Interestingly, though, neither Watson and Balon (1984) nor Welcomme (1985) found that tropical rivers were more productive than temperate rivers. In the former, production was more a function of river fertility than water temperature. Similarly, Schindler (1978) found that eutrophication (total phosphorus) explained most of the variability in the productivity of lakes from different regions. In a recent comprehensive study of production of fish populations in lakes, however, Downing and Plante (1993) found that production was a function of mean biomass and fish size, but it was also correlated with water temperature, primary productivity, lake phosphorus concentration, and pH. Environmental factors not considered in this study probably affected production as well. However, accounting for the environmental factors that contributed to the range of production values observed within each of the lake and river data sets was beyond the objectives of this paper. In future it would be informative to consider these environmental factors and also to compare estimates of river and lake production from locations of the same latitude with similar geological and catchment area characteristics.

Despite the above limitations, it is tempting to generalize about the relative productivity of different freshwater habitats. This study has shown that average fish production was least in lakes, averaging 82 $kg \cdot ha^{-1} \cdot year^{-1}$, and highest in rivers, averaging 273 $kg \cdot ha^{-1} \cdot year^{-1}$. Between these two extremes, fish production would vary depending on the type of habitat and other factors. Lake production is expressed over the entire lake area; production would be higher in the littoral habitats of the lakes. Few estimates of fish production in littoral habitats have been made. Fish production in manmade reservoirs is higher than in lakes. Reservoirs can be considered to be an intermediate habitat type between natural lakes and rivers (Regier and Henderson 1973; Carline 1986). Because of the influence of the river inflow and the high water turnover rates, reservoirs are usually more productive than natural lakes. Jenkins (1982) found that fish yield in reservoirs in the United States was greater than in north temperate lakes. Biomass in 107 reservoirs in United States averaged 208 $kg \cdot ha^{-1}$ (Jenkins 1967). Assuming a P/B ratio of about 1.0, intermediate between lakes and rivers (Table 1), this biomass would indicate production in reservoirs is approaching the mean production we observed for rivers. For these general categories of freshwater habitat, therefore, the production rate of fishes averaged between about 50 and 300 $kg \cdot ha^{-1} \cdot year^{-1}$.

Boudreau and Dickie's (1989) regression method for estimating fish production is a useful tool for resource managers. Canada's Policy for the Management of Fish Habitat (Anonymous 1986) attempts to achieve an overall net gain in the productive capacity of fish habitats. Achievement of this objective requires that the current productive capacity of habitats be conserved and also that damaged habitats be restored. Operationally, this policy emphasizes that habitat biologists must be able to measure productive capacity and effectively predict the effect of habitat alterations on productive capacity. Available production data provides estimates of average production for a variety of fluvial and lacustrine habitats. Reasonable estimates of population or community production can be estimated using Boudreau and Dickie's equation and information on fish biomass and average fish size. Studies at the community level are particularly desirable because total biomass is more likely to reflect carrying or productive capacity. Community biomass can remain remarkably constant over time, despite temporal changes in species composition and size structure (Dickie et al. 1987*a*), and thus few estimates of biomass may be needed to provide at least approximate estimates of productive capacity. Information on the size structure of the biomass will make more accurate estimates of productive capacity possible.

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Appendix 1

Fish production in lakes.

Lake	Biomass (kg·ha^{-1})	Production (kg·ha^{-1}·year^{-1})	Species richness (no.)	Density (no.·ha^{-1})	TPhos (µg·L^{-1})	References
Alinen Mustajarvi, Finland	73.0	30.3	3	4 050	39.3	Rask and Arvola 1985; Downing et al. 1990
Batorin, USSR	163.0	83.0				Winberg et al. 1972; Downing et al. 1990
Big Turkey, Canada	7.3	3.8	9	2 054	4.6	Kelso 1988; Downing et al. 1990
Botjarn, Sweden	30.3	17.3			13.5	Nyberg 1979; Downing et al. 1990
Char, Canada	9.2	2.0	1	356	3.8	MacCallum and Regier 1984; Downing et al. 1990
Dalnee, USSR	197.0	398.0	3			Krogius et al. 1972; Downing et al. 1990
Demenets, USSR	372.2	228.5	7	55 730		Gulin and Rudenko 1973; Downing et al. 1990
Horkkajarvi, Finland	32.2	15.5	2	1 330	114.5	Rask and Arvola 1985; Downing et al. 1990
La Luisa, Cuba	325.7	276.9				Holcik 1970; Downing et al. 1990
Little Turkey, Canada	11.2	4.7	11	296	5.0	Kelso 1988
Marion, Canada	28.9	21.5	2	1 615		Efford 1972; Downing et al. 1990
Myastro, USSR	125.0	64.0				Winberg et al. 1972; Downing et al. 1990
Nakuru, Kenya	59.4	145.8			9850.0	Vareschi and Jacobs 1984 and others, as cited in Downing et al. 1990
Naroch, USSR	81.0	44.0				Winberg et al. 1972; Downing et al. 1990
Heimdalsvatn, Norway	19.8	14.7			14.7	Kloster 1978 and others; as cited in Downing et al. 1990
Red Deer, Canada	11.6	30.8		2 033		Chadwick 1976
Sabanilla, Cuba	311.7	220.5				Holcik 1970; as cited in Downing et al. 1990
Vitalampa, Sweden	20.2	9.8			16.5	Nyberg 1979; as cited in Downing et al. 1990
Washington, U.S.A.	93.4	89.4			34.8	Edmondson 1977 and others; as cited in Downing et al. 1990
Wishart, Canada	24.4	5.7	8	111	5.0	Kelso 1988; Downing et al. 1990
Long Pond, Canada	277.8[a]	85.7	22	20 320		Mahon and Balon 1977
Sawlog, Canada	49.3[a]		8	1 945		Fraser 1981
Pine, Canada	35.2[a]		8	3 414		Fraser 1981

Appendix 1 *(concluded).*

Lake	Biomass (kg·ha^{-1})	Production (kg·ha^{-1}·year^{-1})	Species richness (no.)	Density (no.·ha^{-1})	TPhos (µg·L^{-1})	References
Lonesome, Canada	50.8[a]		8	3 480		Fraser 1981
Redpine, Canada	50.7[a]		9			Fraser 1981
19, Ontario, Canada	29.5	8.6	4.5	459	10.2	Kelso and Johnson 1991; mean of 19 lakes
Shagg, U.S.A.	7.3[a]		6	530		Rupp and DeRoche 1965
Coleback, U.S.A.	19.2[a]		5	2 296		Rupp and DeRoche 1965
Hills, U.S.A.	11.5[a]		5	1 160		Rupp and DeRoche 1965
Iso Mustajarvi	19.5[b]		4	1 432		Lappalainen et al. 1988
Ruuttanajarvi	51.7[b]		1	3 358		Lappalainen et al. 1988

Note: Data on biomass (*B*), production (*P*), species richness, and fish density are summarized from a variety of lakes as reported in the literature. Information on total phosphorus levels (TPhos) are also given if available.

[a] Standing biomass at the time of sampling.

[b] Biomass of *Perca fluviatilis* (the dominant species) only.

Appendix 2

Fish production in rivers.

River	*B* (kg·ha^{-1})	*P* (kg·ha^{-1}·year^{-1})	Species richness (no.)	Density (no.·ha^{-1})	TPhos (µg·L^{-1})	References
Amazon, Venezuela	1600.0	2800.0				Bayley 1983; Welcomme 1985
Thames, England		1980.0				Mann et al. 1972
Bere, England	161.2	270.0	9	130 000		Mean of 3 sites; Mann 1971
Tarrant, England	198.0	596.0	9	778 000		Mann 1971
Devil's, England	95.0	198.0	8	64 000		Mann 1971
Docken's, England	75.0	140.0	7	25 000		Mann 1971
Black Brows, England	59.0	100.0	3	24 000		Le Cren 1969
Kingswell, England	51.0	74.0	2	9 000		Le Cren 1969
Hall, England	129.0	52.0	1	7 000		Le Cren 1969
Appletreeworth, England	62.0	30.0	1	4 000		Le Cren 1969
Hinau North, New Zealand		142.8	1			Hopkins 1971
Hinau Main, New Zealand		241.7	4			Hopkins 1971
Hinaki, New Zealand		735.4	4			Hopkins 1971
Swan, Canada	124.4	174.5	16	50 206		Mahon and Balon 1985; Watson and Balon 1984
Carroll, Canada	274.8	280.3	15	115 125		Mahon and Balon 1985; Watson and Balon 1984
Hopewell, Canada	228.4	243.5	15	154 163		Mahon and Balon 1985; Watson and Balon 1984
Irvine, Canada	149.9	226.4	15	208 253		Mahon and Balon 1985; Watson and Balon 1984
Ellis, Canada	376.0	375.4	17	100 036		Mahon and Balon 1985; Watson and Balon 1984
Upper Carrol, Canada	278.7	379.1		205 183		Mahon and Balon 1985
Mud CK, Canada	127.5	217.6		61 667		Mean of 3 sites; Portt 1980 (as cited in Mahon and Balon 1985)
North Branch, Canada	217.5	500.6		131 000		Mahon and Balon 1985
Irvine2, Canada	187.2	297.2		283 537		Halyk and Balon 1983; Mahon and Balon 1985
Struga, Poland	111.2	110.3	2	39 247		Mahon and Balon 1985; Watson and Balon 1984
Lubrzanka, Poland	241.0	128.8	15	17 230		Mahon and Balon 1985; Watson and Balon 1984

Appendix 2 *(continued)*.

River	*B* (kg·ha^{-1})	*P* (kg·ha^{-1}·year^{-1})	Species richness (no.)	Density (no.·ha^{-1})	TPhos (μg·L^{-1})	References
Warkocz, Poland	307.5	161.4	7	50 058		Mahon and Balon 1985; Watson and Balon 1984
Bobrza, Poland	50.2	83.4	4	23 698		Mahon and Balon 1985; Watson and Balon 1984
Utrata1, Poland	310.5	807.2	3	33 370		Penczak 1981; Mahon and Balon 1985
Utrata2, Poland	142.5	228.0	8	33 559		Penczak 1981; Mahon and Balon 1985
Utrata3, Poland	86.6	329.0	4	19 595		Penczak 1981; Mahon and Balon 1985
Utrata4, Poland	45.6	168.7	5	93 200		Penczak 1981; Mahon and Balon 1985
Utrata5, Poland	10.8	57.2	8	6 581		Penczak 1981; Mahon and Balon 1985
Utrata6, Poland	40.9	147.3	5	46 055		Penczak 1981; Mahon and Balon 1985
Zalewka1, Poland	49.8	54.8	5	6 230		Penczak 1981; Mahon and Balon 1985
Zalewka2, Poland	42.5	76.5	6	46 327		Penczak 1981; Mahon and Balon 1985
Zalewka3, Poland	38.4	30.7	5	6 484		Penczak 1981; Mahon and Balon 1985
Wolborka, Poland	37.4	35.3		3 714		Penczak et al. 1982; Mahon and Balon 1985
Kejin1, Malaysia	173.1	261.5	23	206 021		Watson and Balon 1984
Kejin2, Malaysia	71.0	98.3	19	53 873		Watson and Balon 1984
Lawa1, Malaysia	30.5	39.3	25	15 866		Watson and Balon 1984
Lawa2, Malaysia	21.3	33.3	29	9 789		Watson and Balon 1984
Kaha, Malaysia	38.5	47.5	32	11 389		Watson and Balon 1984
Bulu, Malaysia	21.5	26.1	16	8 262		Watson and Balon 1984
Payau, Malaysia	27.1	31.2	23	7 282		Watson and Balon 1984
Philip, Canada	36.0	53.0	6	13 300		Randall et al. 1989; R.G. Randall (personal communication)
Jaruma, Spain	233.4	456.2	6	45 458		Mean of 3 sites; Lobon-Cervia and Penczak 1984
Big Springs, U.S.A.	84.2	118.0	4			Goodnight and Bjornn 1971; Welcomme 1985
Lemhi, U.S.A.	212.0	136.0	5			Goodnight and Bjornn 1971; Welcomme 1985
Mesta, Bulgaria	80.2	80.3	5.6	6 638		Mean of 5 sites; Penczak et al. 1985
Speed, Canada	15.4	29.4	11	24 547		Mean of 3 sites; Mahon et al. 1979
Kafue, Zambia	520.0	618.0				Kapetsky 1974; cited in Welcomme 1985
Deer, U.S.A.	84.7	160.0	4			Chapman 1965; Welcomme 1985
Needle Branch, U.S.A.	45.9	90.0	3			Chapman 1965; Welcomme 1985
Clemons Fork 1, U.S.A.	54.9	83.5	1			Lotrich 1973; Welcomme 1985
Clemons Fork 2, U.S.A.	63.6	105.5	8			Lotrich 1973; Welcomme 1985
Clemons Fork 3, U.S.A.	71.5	77.2	15			Lotrich 1973;Welcomme 1985
15, Florida, U.S.A.	95.1				194.0	Hoyer and Canfield 1991[a]
19, Vermont, U.S.A.	7.4				7.2	Hoyer and Canfield 1991[a]
2, Washington, U.S.A.	52.0				81.5	Hoyer and Canfield 1991[a]

Appendix 2 *(concluded)*.

River	*B* (kg·ha^{-1})	*P* (kg·ha^{-1}·year^{-1})	Species richness (no.)	Density (no.·ha^{-1})	TPhos (μg·L^{-1})	References
10, Ontario, Canada	104.2				122.1	Hoyer and Canfield 1991[a]
20, Wyoming, U.S.A.	110.8				25.4	Hoyer and Canfield 1991[a]
1, Missouri, U.S.A.	57.0				11.0	Hoyer and Canfield 1991[a]
12, Iowa, U.S.A.	251.0				307.3	Hoyer and Canfield 1991[a]

Note: Estimates of biomass (*B*), production (*P*), species richness, and fish density in several rivers as reported in the literature.

[a] Biomass and phosphorus values are averages for the number of rivers indicated. See also additional references cited by Hoyer and Canfield 1991.

Pacific salmon and Pacific herring mortalities in the Fraser River plume caused by river lamprey (*Lampetra ayresi*)

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Abstract: River lamprey (*Lampetra ayresi*) enter the Strait of Georgia from the Fraser River and feed almost exclusively on Pacific herring (*Clupea harengus*) and salmon (*Oncorhynchus* spp.). Although the major prey of river lamprey is Pacific herring, the greater effect of lamprey predation was on the populations of chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon. In 1990 and 1991, river lamprey killed a minimum of 20 million and 18 million chinook salmon, respectively, and a minimum of 2 million and 10 million coho salmon in the same years. In 1991, river lamprey in the Fraser River plume killed an equivalent of approximately 65 and 25% of the total Canadian hatchery and wild production of coho and chinook salmon, respectively. These estimates are probably low because river lamprey also feed in other areas and the abundance estimates are conservative. These high mortality rates indicate that river lamprey predation must be considered as a major source of natural mortality of chinook and coho salmon in the Strait of Georgia.

Résumé : La lamproie à queue noire (*Lampetra ayresi*) quitte le Fraser pour entrer dans le détroit de Géorgie et se nourrit presque exclusivement de hareng du Pacifique (*Clupea harengus*) et de saumon (*Oncorhynchus* spp.). Bien que sa principale proie soit le hareng du Pacifique, c'est sur les populations de quinnat (*O. tshawytscha*) et de coho (*O. kisutch*) que l'effet de la lamproie se fait le plus sentir. En 1990 et 1991, les lamproies à queue noire ont tué respectivement un minimum de 20 millions et de 18 millions de quinnats et un minimum de 2 millions et de 10 millions de cohos. En 1991, les lamproies à queue noire présentes dans le panache du Fraser ont tué l'équivalent d'environ 65 et 25 % respectivement de la production totale canadienne de coho et de quinnat (poissons d'élevage et poissons sauvages combinés). Ces estimations sont probablement faibles, du fait que la lamproie à queue noire se nourrit aussi dans d'autres régions et nos estimations de l'abondance sont conservatrice. Ces taux élevés de mortalité indiquent que la prédation par la lamproie à queue noire doit être considérée comme une source importante de mortalité naturelle du quinnat et du coho dans le détroit de Géorgie.

[Traduit par la Rédaction]

Introduction

Marine mortality for all Pacific salmon species (*Oncorhynchus* spp.) generally exceeds 50% and frequently exceeds 90%. Most of this mortality is believed to occur shortly after the salmon smolts enter salt water (Groot and Margolis 1991; Pearcy 1992), although there is little scientific evidence to support this belief. Predation is the obvious source of the early marine mortality because even if food is scarce, "starving" salmon are believed to be killed by predators, but again there is little scientific support for this hypothesis. It is important to understand the sources of early marine mortality of salmon because the effort and cost of increasing smolt production in fresh water should be assessed with an understanding of how much of this increased production might actually survive in the marine environment.

In an earlier study, spiny dogfish (*Squalis acanthias*) were identified as important predators of young Pacific salmon entering the Strait of Georgia (Beamish et al. 1992). Large numbers of spiny dogfish moved into shallow water at almost the exact time that chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon smolts started moving into salt water. Even though predation rates of spiny dogfish on salmon were small, the large numbers of spiny dogfish were considered to be an important source of early marine mortality of salmon. A second important predator of Pacific salmon and Pacific herring (*Clupea harengus*) in the Strait of Georgia is the river lamprey (*Lampetra ayresi*) (Roos et al. 1973; Beamish 1980).

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The river lamprey has a restricted distribution but because of its abundance and feeding behaviour, it is potentially an important source of marine mortality of Pacific salmon in the Strait of Georgia. Although *L. ayresi* has been reported in the Skeena River (Withler 1955), it is most common in the Fraser River where it has been shown to be the dominant organism by weight in the bottom sediments of the 160 km section of river from the city of Hope to the river mouth (Beamish and Youson 1987). Newly metamorphosed *L. ayresi* enter the Strait of Georgia after the lumen of the oesophagus opens, and the lamprey is able to osmoregulate in salt water (Beamish and Youson 1987). The migration into salt water occurs from April to July during maximum Fraser River discharge. River lamprey begin feeding almost immediately after entering the ocean. In earlier studies (Beamish 1980; Beamish and Youson 1987), we speculated on the impact of lamprey predation on salmon and herring. In this study, we attempt to measure the amount of species specific salmon and herring mortality in the Fraser River plume caused by river lamprey predation.

Methods

Lamprey that feed on other animals are generally considered to be parasites because their hosts may not be killed by the feeding event. The river lamprey, however, feeds by removing flesh from the host and should be considered to be a predator rather than a parasite. Determining the number of prey of each species killed by river lamprey predation required a population estimate of lamprey in the plume, an estimate of the total number of prey, an estimate of the percentage of each prey species in the lamprey diet, and estimates of the prey mortality that resulted from each lamprey attack. When making these determinations, we ensured that any approximations were conservative. Estimates of prey mortality, therefore, should be conservative.

Abundance of river lamprey, Pacific herring, and salmon

A trawl survey of the surface waters of the Fraser River plume and adjacent waters was conducted in 1989, 1990, and 1991. The trawl net had a 4 × 4 m opening with stretched mesh side panels varying from 9.0 cm at the mouth to 0.5 cm in the cod end (Whitehouse and Levings 1989). The top of the trawl was held at the surface using floats attached to the head-rope. The net was kept open using 1.24 × 0.4 m midwater trawl doors. The net was towed off the stern at a speed of approximately 2 kn for approximately 30 min. All river lamprey captured were counted, measured, and preserved in 10% formalin. Pacific herring and salmon were examined for lamprey wounds and measured. All salmon were preserved in 10% formalin so that their identification could be confirmed in the laboratory using standard taxonomic characteristics.

Comparisons of lamprey catches inside and outside the Fraser River plume were made by standardizing the length of sets to 1 km of water strained and comparing average catch in the two areas. Estimates of fish abundance in the plume were obtained by multiplying the total catch by the plume area and dividing by the amount of water strained. The amount of water strained was estimated by multiplying the width of the net by the total distance trawled. Salinity measurements indicated that the plume varied in thickness from several metres to several centimetres thick. Our nets fished about 4 m deep, but we used surface area fished rather than volume because the variation in the thickness of the plume made it impractical to estimate the depth of the plume during the study. We assumed that the net caught all fish in the path of the net opening.

June is the period of maximum discharge of the Fraser River and the time when most young juvenile river lamprey enter the Strait of Georgia (Beamish 1980). The shape, depth, and area of the plume is influenced by Fraser River discharge, tide, and wind and is continuously changing (St. John et al. 1992). Because of the changing shape of the plume and because it is not possible to obtain area estimates daily, we estimated the plume's average size from the generalized surface salinity pattern determined by Waldichuk (1957) for June 1950. In 1950, the total annual discharge was about average for this century (Beamish et al. 1994) and less than in 1990 and 1991 (Environment Canada 1990). Harrison et al. (1991) identified the plume area as having a surface salinity from 10 to 15%. We selected a salinity of 10% as the boundary of the plume to ensure we used a conservative estimate of the average size of the plume in June.

In 1989 and 1990, sets were made randomly throughout the plume, and a smaller number were made just outside the plume boundary. The survey design was modified to a random stratified design in 1991. Fewer trawls were set in the centre of the plume, which had lower, more consistent catches of river lamprey in the previous years, and more trawls were made just inside the outer boundary of the plume. This area had higher, but more variable, catches of river lamprey in previous years. A small number of trawls were made immediately outside the plume boundary, which could usually be distinguished by the abrupt transition from silty plume water to the clearer water of the Strait of Georgia. Temperature and salinity were measured at the end of each set to verify our position within the plume. The abundance of Pacific herring and salmon in the plume in 1990 and 1991 was estimated using the swept-area procedure used for river lamprey.

We speculated that natural mortality of lamprey had only minor effects on our abundance estimates because they were made about midway during the lamprey feeding period. There is no estimate of natural mortality of lamprey in salt water and therefore, we chose an annual natural mortality of $M = 0.4$. This mortality rate, commonly observed for marine fishes, was applied to the time remaining in the feeding period after the lamprey population size was estimated.

Intestinal contents of river lamprey

In 1991, the percentage of salmon and Pacific herring in the diet of river lamprey was estimated from the intestinal contents. Intestinal contents were determined in the laboratory from a random sample of 103 of 786 lamprey that were collected in 1991 and preserved in 10% formalin. The body weight and length of the preserved lamprey were measured before dissection. The entire intestine was then removed and weighed separately. Intestines with contents

Fig. 1. River lamprey gut volume versus gut length for 1976 (●) and 1991 (Δ). Line fitted to combined data sets is gut volume + $e^{(2.94 + 0.174 \times \text{length})}$.

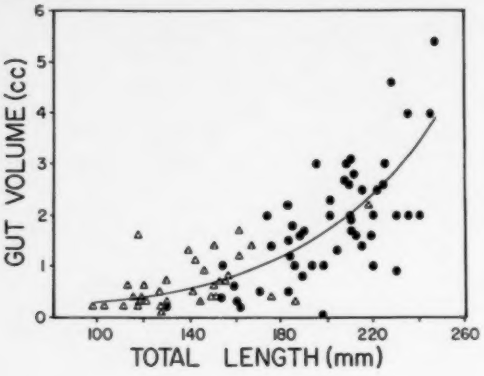

Table 1. Species and numbers captured in the Fraser River plume during 1989, 1990, and 1991 trawl surveys.

	1989	1990	1991
River lamprey	181	518	899
Chinook salmon	43	84	205
Chum salmon	2	49	141
Coho salmon	21	15	61
Sockeye salmon	8	47	130
Pink salmon	4	23	6
Pacific herring >100 mm	262	632	4062
Pacific herring ≤100 mm	558	2756	1528
Stickleback	13	94	53
Sandlane	11	13	130
Starry flounder	0	11	3
Smelt	64	7	17
Other	4	12	36

less than 0.1 g were considered to be empty. Scales from the intestines were identified as salmon, Pacific herring, or not identifiable.

Wounding studies

All herring and salmon caught during the trawl surveys were examined for lamprey wounds. In 1990, wounds were recorded and in 1991 wounds were counted and scars (healed wounds) were recorded. In both years, the percentage of the catch of each species with wounds was calculated. The wounding percentages for the various salmon species was used to estimate the species specific mortality. The type of wound was important because a high percentage of scarred prey would indicate that prey survived the lamprey feeding events, where as a high percentage of fresh wounds probably resulted when some prey escaped in the trawl.

Number of prey attacked

In the plume, very few scarred prey were found, indicating that the relatively large wounds caused by lamprey were fatal to the hosts. Therefore, we assumed that an average feeding event would ultimately cause the death of the host. Because we could not observe feeding behaviour in the wild, we estimated the number of hosts attacked by estimating the minimal number of feeding events required for river lamprey to grow from the average size when they entered salt water to the estimated terminal size.

The average size at first feeding was 12 cm and 1.8 g (Beamish 1980), and the terminal size was approximately 25 cm and 34 g. This is the size of the only known river lamprey captured during its return to the spawning ground in the Fraser River and is very similar to the average terminal size of 27 cm and 34 g of river lamprey fed in the laboratory (Beamish and Williams 1976; Beamish and Scarsbrook 1979). We used a conversion rate of 29% (Brett and Groves 1979) to estimate the amount of flesh consumed for an average lamprey to grow to 34 g. This high conversion rate was for young, fast-growing, well-fed fish. The rate is less than the 39% conversion rate determined by Farmer (1980) for the landlocked sea lamprey (*Petromyzon marinus*). The 29% conversion rate was selected because the river lamprey feeds by consuming tissue, similar to the fish studied by Brett and Groves (1979), whereas the sea lamprey consumes body fluids. Consumption of body fluids would result in a lower faecal energy loss than the consumption of tissue (Farmer 1980). Beginning at 12 cm and 1.8 g, we estimated the average intestinal volume for this length from Fig. 1. Twenty-nine percent of this volume would be added to the weight (assuming 1 cc (1 mL) of flesh weighs 1 g). Using the length–weight relationship, $W = 1.44 \times 10^{-3}$ L $(\text{cm})^{2.978}$ (Beamish 1980) we estimated the new length and repeated the calculation for the second feeding event. We continued our calculations until the terminal size of 34 g was reached. The number of feeding events was summed and considered to be the minimum average number of hosts that would be attacked.

We used a second method to estimate the number of hosts attacked that did not fix the growth rate but fixed initial and terminal sizes. We used the average daily growth determined from laboratory feeding studies to estimate the number of days it took lamprey to grow from the size at entry into salt water to their terminal size (27 cm and 34 g). Assuming that a lamprey feeds once a day (Beamish and Williams 1976), we considered the number of days to represent the average number of hosts attacked and killed and used this second estimate to check the first estimate that we considered to be more reliable.

Feeding studies

River lamprey were collected from the southern Strait of Georgia during June 1988. They were transported to the Pacific Biological Station, Nanaimo, British Columbia, and held in aquaria at ambient temperature and supplied with continuously flowing salt water (28%). Rock and gravel substrate was placed in the bottom of the tanks to provide the lamprey with areas of shelter. Live Pacific herring were placed in the tanks as a food source.

An experiment, to study growth of river lamprey, was conducted from August 9 to September 20, 1988. Eighteen

Table 2. Numbers and percentages of lamprey wounds on Pacific herring and salmon.

	1990			1991		
	Catch No.	Wound No.	Wound (%)	Catch No.	Wound No.	Wound (%)
Pacific herring ≥100 mm	632	123	19	4062	641	16
Chinook salmon	83	11	13	205	23	11
Chum salmon	51	8	16	141	13	11
Coho salmon	17	1	14	61	13	9
Sockeye salmon	44	0	0	130	15	21
Pink salmon	22	1	5	6	1	17
Total salmon	217	21	10	543	65	12
Total	849	144	17	6585	706	11

river lamprey were measured and weighed and then placed in a 350-L tank with 50 Pacific herring. Dead Pacific herring were removed and replaced daily. The herring were measured for fork length, and the number of the lamprey wounds were recorded. Lamprey remaining at the termination of the experiment were measured for length and weight.

Table 3. Number of wounds on Pacific herring and salmon in 1991.

	Single wound	Multiple wound
Pacific herring (≥100 mm)	552	89
Pacific salmon (all species)	61	4

Results

River lamprey density and abundance

Estimates of the abundance of river lamprey were made using 1990 and 1991 data. The 1989 survey captured 181 lamprey in 85 sets. We used this information to design the 1990 and 1991 surveys and not for population estimates because the sampling locations in 1989 were selected as part of another study and did not sample the plume effectively. From July 4–26, 1990, 63 sets inside the plume caught 518 lamprey, and 8 sets outside the plume caught 5 lamprey or 7.9 and 1.0 lamprey/km, respectively. From June 10 – July 12, 1991, a total of 87 sets inside the plume resulted in a catch of 899 river lamprey or 10.3 lamprey/km, and 5 sets outside the plume resulted in a catch of 4 lamprey of 1.4 lamprey/km. In 1990 and 1991, the ratio of river lamprey in the Fraser River plume and outside the plume was 7.9:1 and 7.4:1, respectively.

Abundance estimates were calculated for the Fraser River plume using the total catch for the total area surveyed of 0.26 km^2 in 1990 and 0.35 km^2 in 1991. The number of lamprey caught within the survey area was then calculated for the area of the plume of 1506 km^2 (about 22% of the total area of the strait). The abundance of lamprey in the plume in 1990 and 1991 was 3 million (± 700 000) and 3.9 milion (± 963 000), respectively.

Catches of fishes other than lamprey

Pacific herring and all five species of Pacific salmon in the Strait of Georgia were caught in the trawls (Table 1). Other species were much less abundant. Catches of Pacific herring from the 1989, 1990, and 1991 surveys were the largest of all species captured (Table 1). Catches of Pacific herring were separated into two length-classes because Pacific herring less than 100 mm tended to be young-of-the-year (40–90 mm, average 77 mm) and more susceptible to damage from the trawl net, making it difficult on occasion to distinguish between net damage and lamprey wounds.

All salmon except pink salmon were more abundant in the catches in 1991 than in 1990 (Table 1). The average catch rate of all five species in the plume in 1990 and 1991 was 3.46 and 6.03 salmon/km, respectively. This compares to an average catch rate of 0.27 and 2.11 salmon/km outside the plume in 1990 and 1991, respectively. In 1991, the catch of herring >100 mm (average 144 mm) was almost five times higher than in 1990 (average 133 mm). The average lengths of Pacific salmon caught during 1990 were as follows: chinook, 110 mm (±31); coho, 144 mm (±30); chum, 109 mm (±15); pink, 102 mm (±14); and sockeye, 68 mm (±15). The average lengths in 1991 were as follows: chinook, 89 mm (±22); coho, 130 mm (±47); chum, 103 mm (±20); pink, 78 mm (±13); and sockeye, 68 mm (±18).

Lamprey wounds on Pacific herring and salmon

Wounds caused by lamprey attacks were easily separated from other marks. Lamprey wounds ranged from circular depressions to longitudinal gouges. There was a loss of scales at the edges of the wound, and occasionnaly there were puncture holes anterior of the position of first attachment. Other marks, which were rare, were not associated with such extensive descaling and with the loss of flesh.

The combined percent of Pacific herring and salmon with wounds was similar in 1990 and 1991 (Table 2). Wounding percentages for chinook, coho, and chum salmon were slightly lower in 1991 than in 1990, but those for sockeye and pink salmon and Pacific herring were higher in 1991 than in 1990. In 1991, multiple wounds were found

Table 4. Estimated numbers of salmon (by species and total) and Pacific herring killed in the Fraser River plume by river lamprey in 1990 and 1991.

	1990 (millions)	1991 (millions)
Chinook salmon	20	18
Chum salmon	15	10
Coho salmon	2	10
Sockeye salmon	0	12
Pink salmon	2	1
Total salmon	39	51
Pacific herring	156	203

on 4 salmon or 6% of those with wounds and on 89 Pacific herring or 14% of those with wounds (Table 3). Only one salmon and nine herring had more than 2 wounds in the 1991 sample. Scars (healed wounds) were rarely observed. In 1991, only 28 or 0.7% of all Pacific herring caught had scars and 6 or 0.9% of all salmon caught had scars.

Laboratory feeding studies

In the feeding experiment to study lamprey growth, the average size of lamprey increased from 15.4 cm (±2.7) and 4.1 g (±2.2) to 23.1 cm (±3.7) and 17.8 g (±10.6) in 42 d. One lamprey died after 6 d and one after 8 d; it was unlikely that they had fed. These lamprey were not replaced and not included in the estimate of average growth in length. In this 42-d period, 128 Pacific herring were killed or had severe wounds that would likely result in death. Almost all of these herring had multiple wounds.

Lamprey intestinal contents

The intestinal contents of the lamprey contained some scales and portions of the internal organs of their prey. Of the 103 intestines examined, 25% were empty and 75% contained food. Of the intestines with food, 43 (56%) had contents that were not identifiable and 34 (44%) contained scales or pieces of scales. Pacific herring scales were found exclusively in 20 intestines, and salmon scales were found exclusively in 8 intestines. The remaining six intestines contained both salmon and herring scales, but in all cases herring scales represented >75% of the scales. These intestines were classified as containing Pacific herring because the percentage of salmon remains was small and our estimate of the percentage of herring and salmon attacked is an approximation. Thus, 26% of the intestinal contents that could be identified were from salmon. In the study by Beamish and Williams (1976), salmon remains were found in 14% of the intestinal content of lampreys. The average of these two studies, therefore, indicated that lamprey prey were 20% salmon and 80% herring and other species. Because very few species other than salmon and herring were caught (Table 1), and only a few had lamprey wounds, we considered the prey to consist only of salmon and Pacific herring.

The volume of intestinal contents from this study and from Beamish and Williams (1976) was compared to lamprey length (Fig. 1). This relationship was needed to estimate the amount of flesh consumed in the methods used to determine the number of prey killed.

Average number of prey killed

The two methods used to estimate the number of prey killed produced very similar estimates. In the first method, using a conversion efficiency, we started with a weight of 1.8 g. Using this method we estimated that it would take 65 feeding events for a lamprey to grow from 1.8 g to its average terminal size of 34 g.

Our second estimate of the number of prey killed, based on the results of the laboratory feeding study, indicated that in 42 d the average lamprey increased in size from 15.4 to 23.1 cm. Knowing that it took 42 days to grow from 15.4 to 23.1 cm, we calculated by linear extrapolation that the average time it would take to grow from 12–25 cm was 74 days. Assuming one prey was killed per day, 74 prey would be killed.

The two methods, therefore, produced estimates of 65 and 74 prey killed. To be conservative, we used the estimate of 65 prey killed.

Estimated mortality of Pacific salmon and herring

Our estimate of lamprey abundance in 1990 was made after about one-third of the average growth had occurred. Thus, there were about 45 d of feeding left until the average lamprey reached the average terminal size. Using our estimate of natural mortality of $M = 0.4$, the total mortality during the remaining feeding period was approximately 144 000 lamprey. A similar estimate for 1991 indicated that approximately 188 000 lamprey would die as a result of natural factors. Because our estimates of lamprey abundance must be considered approximate and because the mortality estimates are relatively small, we chose to use the lamprey abundance estimates without correcting for natural mortality before and after the abundance estimates were made.

The number of salmon killed was determined as the lamprey population × 65 prey killed × 0.2% of the prey. An estimated 39 million Pacific salmon of all species were killed in 1990 and 51 million salmon were killed in 1991 (Table 4). Using the wounding percentages for each salmon species (Table 2) as an indication of the relative deaths for each species, we estimated the total mortality for each species of salmon (Table 4). In both 1990 and 1991, more chinook salmon were killed than any other Pacific salmon species. Chum salmon deaths were also high in both years. In 1991, both coho and sockeye salmon deaths were considerably higher than in 1990. Pink salmon deaths were the lowest, especially in 1991 when there were few smolts leaving the Fraser River. In 1990 and 1991 there were an estimated 156 million and 203 million Pacific herring larger than 100 mm killed directly or indirectly by river lamprey (Table 4).

Discussion

Relative importance of lamprey predation

In this study we estimated that river lamprey predation in the Fraser River plume killed 39 million salmon in 1990 and

51 million salmon in 1991. Chinook salmon were preyed upon in the highest numbers with approximately 20 million killed in 1990 and 18 million killed in 1991. These estimates can be put into perspective by comparing them with hatchery releases. In 1990 and 1991, all the Canadian hatcheries releasing chinook salmon directly into the Strait of Georgia and into the Fraser River and then into the Strait of Georgia produced a total of approximately 33 million and 34 million smolts, respectively. The mortality caused by river lamprey in the Fraser River plume in 1990 and 1991 is equivalent to approximately 62 and 53%, respectively, of these releases. There is evidence that the numbers of wild and hatchery reared chinook smolts that entered the Strait of Georgia at this time may have been about equal (R.J. Beamish, Pacific Biological Station, Nanaimo, BC V9R 5K6, unpublished data). If this approximation is correct, the mortality in the plume by river lamprey accounts for about 25–30% of the total marine mortality of all chinook salmon entering the Strait of Georgia from Canadian sources.

The mortality estimates of coho for the same period were not as consistent as those for chinook salmon. The 1990 estimate was approximately 2 million coho and the 1991 mortality estimate was approximately 10 million coho. In 1990 and 1991, all Canadian hatcheries that released coho salmon directly or indirectly (from the Fraser River) into the Strait of Georgia produced 7.6 million and 7.7 million smolts, respectively. Again, very preliminary evidence is that the abundance of hatchery and wild coho was about equal when they first enter salt water during the study period (R.J. Beamish, unpublished data). Thus, in 1990, coho mortality in the plume is equivalent to 26% of the total hatchery release into the Strait of Georgia or about 13% of all coho smolts entering the Strait. Coho mortality in 1991, in the plume only, is equivalent to 130% of the total hatchery release into the Strait of Georgia or about 65% of the total mortality of all coho smolts entering the Strait of Georgia.

Estimates of chum salmon smolt production entering the Strait of Georgia are difficult to determine. However, if we use escapements to the Fraser River and other rivers entering the Strait of Georgia of 650 000 and 1.3 million for 1989 and 1990, respectively (Salmon Stock Assessment Data Base, Pacific Biological Station, Nanaimo, BC V9R 5K6), a sex ratio of 1:1, average egg production of 3350 (Salo 1991), and egg-to-smolt survival of 10%, then approximately 108 million and 214 million chum smolts were produced in 1990 and 1991, respectively. Our estimates of chum salmon mortality in 1990 and 1991 of approximately 14.9 million and 10.1 million smolts would represent 13.7 and 4.7% of the total smolt production of chum salmon from the rivers flowing into the Strait of Georgia, and therefore, would not be considered to be a major source of early marine mortality.

We are uncertain why there was an absence of sockeye salmon mortality in 1990 and a large estimate of mortality in 1991. It may appear that we misidentified chum and sockeye salmon, but our identifications were confirmed in the laboratory using standard taxonomic measurements thus we concluded that misidentifications were not the explanation. A possible explanation is that because the abundance of smolts in 1991 was greater than in 1990 (Salmon Stock Assessment Data Base, Pacific Biological Station, Nanaimo, BC V9R 5K6), we did not have a large enough sample to observe the presence of wounds.

The relative importance of lamprey predation on sockeye smolts can be estimated by calculating the approximate number of smolts that went to sea in 1991 from the Fraser River. If we use an egg-to-smolt survival estimate of 10.09% (Foerster 1968), a sex ratio of 1:1, and an average egg production of 3274 (Foerster 1968), the 3 million sockeye salmon (Salmon Stock Assessment Data Base, Pacific Biological Station, Nanaimo, BC V9R 5K6) that escaped to spawn would produce about 511 million smolts in 1991. Our estimates of sockeye salmon mortality in 1991 of approximately 11.7 million is 2.3% of the estimated smolt production in the Fraser River. Lamprey predation on sockeye, therefore, does not appear to be a major source of early marine mortality in the Fraser River plume.

Virtually all pink salmon spawn in odd years in the Fraser River drainage, with the young pink going to sea in even years (Neave 1966). The estimated number of pink salmon deaths were low relative to the other species, even though in even numbered years pink salmon smolts may be the most numerous of all the species of salmon smolts. We conclude that our study did not identify lamprey predation as a major source of pink salmon smolt mortality in the Fraser River plume.

During the spawning season, Pacific herring move into the Strait of Georgia from offshore (Taylor 1964; Hourston and Haegele 1980). In recent years, the estimated average biomass of spawning Pacific herring has been about 50 000 t (range from about 30 000 to 70 000 t (V. Haist, Pacific Biological Station, Nanaimo, BC V9R 5K6, personal communication). Assuming eight herring weigh about 1 kg (Hart 1973), there would be about 500 million spawning fish. The estimates of Pacific herring mortality (larger than 100 mm) were 156 million in 1990 and 203 million in 1991. This mortality is mainly on 1 year olds, not the spawning 3 year old and older fish. Nevertheless, the estimates of herring mortality caused by lamprey are sufficiently large to conclude that lamprey predation could be an important source of marine mortality of Pacific herring in the Strait of Georgia.

In this study, we examined the mortality in the Fraser River plume only. We observed feeding outside the plume and we know that feeding river lamprey occur elsewhere in the strait (Beamish 1980). Abundance estimates of river lamprey were made in two earlier studies. In the 1976 study, the abundance of river lamprey was estimated using the area fished by a purse seine. Minimum and maximum abundance estimates were made based on the minimum and maximum area that could be fished by a purse seine. The maximum abundance estimate was based on the area of the seine when the lamprey would be unlikely to swim through the meshes (Beamish and Williams 1976). The maximum population estimate in 1975 was 6.2 million river lamprey and the minimum was about 10% of this number.

A second estimate of abundance was made in 1979 using gear that captured young lamprey in the Fraser River as they moved into salt water (Beamish and Youson 1987).

This method was based on the volume of river discharge strained and produced a population estimate of 6.5 million river lampreys. The two estimates in this study of 3 million in 1990 and 3.9 million in 1991 are lower than the earlier estimates but are for the plume area only. Considering the difficulty involved in estimating the abundance of any fish and particularly an eel-shaped fish that is difficult to catch, the four abundance estimates are quite similar, even though the 1990 and 1991 estimates are for the plume only. We have no reason to conclude that the abundance of lamprey is constant from year to year, but we can conclude that the mortality estimates based on the population of lamprey in the plume only, will be conservative, possibly accounting for approximately one-half to two-thirds of the actual deaths.

The problem of lamprey predation is not unique to the Strait of Georgia. The landlocked sea lamprey has been a source of mortality for salmonids in the Great Lakes and considerable effort has been expended to reduce the impact of lamprey-caused mortality (Smith and Tibbles 1980). Reduction of lamprey abundance in the Great Lakes has increased the abundance of salmonids and it is possible that a program to reduce river lamprey abundance could improve the natural survival of chinook and coho salmon in the strait. Any program that attempted to control river lamprey abundance should include studies of the other key factors that regulate the abundance of chinook and coho in the strait so that the effect of a control program can be evaluated.

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REVIEW / SYNTHÈSE

Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems

Robert France

Abstract: Existing data conflict as to the capability of stable isotope analysis (SIA) for distinguishing carbon pathways in stream ecosystems. I compiled 803 published measurements of $\delta^{13}C$ for allochthonous litter and both lotic attached algae and consumers to search for general patterns that transcend individual studies. Half of the fishes and 30% of the invertebrates exhibited $\delta^{13}C$ values outside the range that could be ascribed to allochthonous litter. The enormous variability in attached algal $\delta^{13}C$ from −40 to −20‰ (mean ± SD = −29 ± 4) completely enveloped that observed for terrestrial leaf litter (mean = −28 ± 1‰). Therefore, for 50% of the fishes and 70% of the invertebrates it was impossible to discriminate between allochthonous and autochthonous carbon dependency. Faunal $\delta^{13}C$ did not change in any quantitatively predictable manner with removal of riparian trees. Three conclusions are possible from this secondary analysis: (1) autotrophic pathways within forested headwaters are much more important to lotic foodwebs than would be suggested by their particulate inputs alone; (2) the great variability in attached algal $\delta^{13}C$ may often preclude use of SIA for identifying carbon pathways in stream ecosystems; thus (3) the utility of carbon SIA in understanding anthropogenic alterations to the carbon budget of streams is presently minimal.

Résumé : Les données existantes se contredisent quant à l'aptitude de l'analyse des isotopes stables à distinguer les voies du carbone dans les écosystèmes lotiques. J'ai compilé 803 mesures publiées de $\delta^{13}C$ concernant la litière allochtone, ainsi que les algues fixées du milieu lotique et leurs consommateurs, pour essayer d'établir des profils généraux qui transcendent les études individuelles. La moitié des poissons et 30% des invertébrés présentaient des valeurs de $\delta^{13}C$ situées à l'extérieur de la fourchette qui pouvait être assignée à la litière allochtone. L'énorme variabilité de $\delta^{13}C$ dans les algues fixées, de −40 à −20‰ (moyenne ± ET = 29 ± 4) débordait entièrement celle qui avait été observée pour la litière feuillue à terre (moyenne = −28 ± 1‰). En conséquence, pour 50% des poissons et 70% des invertébrés, il était impossible de faire une distinction dans la dépendance entre le carbone allochtone et le carbone autochtone. Le $\delta^{13}C$ faunique n'a pas changé de manière quantitativement prédictive après l'élimination des arbres riverains. Trois conclusions peuvent être tirées de cette analyse secondaire : (1) les voies autotrophes dans les eaux d'amont des régions forestières jouent un rôle beaucoup plus important pour les chaînes trophiques lotiques que ne sembleraient l'indiquer leurs seuls apports particulaires; (2) la grande variabilité du $\delta^{13}C$ dans les algues fixées peut souvent empêcher le recours à l'analyse des isotopes stables pour repérer les voies du carbone dans les cours d'eau; donc (3), l'utilité de l'analyse des isotopes stables du carbone pour comprendre les modifications anthropiques du bilan du carbone dans les cours d'eau est actuellement minime.
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Stable isotope analysis…

…"[is] the method with the greatest potential for accurately assessing the [carbon] food base of a stream ecosystem" — Rosenfeld and Mackay (1987)

…"as a survey tool to quickly determine the carbon base of different stream communities is limited" — Rosenfeld and Roff (1992)

Earlier disagreements about the relative importance of allochthonous and autochthonous organic matter for sustaining lotic foodwebs (e.g., Cummins 1974; Minshall 1978) were largely reconciled with the advent of the River Continuum Concept (Vannote et al. 1980), wherein a shifting balance between exogenous and endogenous energy supply is thought to exist along a stream gradient in relation to hydrodynamics and landscape modifications. Headwater streams are thought to be dominated by terrestrial organic inputs from overhanging riparian vegetation. As stream size increases and riparian shading is reduced, more light reaches the water and algal primary production is stimulated. The result is believed to be an inverse relationship between allochthonous energy dependence and stream order (Minshall et al. 1985).

Terrestrial and aquatic plants differ in their mechanisms of carbon uptake during photosynthesis (Keeley and Sandquist 1992), often resulting in dissimilar stable isotope ratios (Peterson and Fry 1987). Because carbon undergoes little further isotopic fractionation during food assimilation (Fry and Sherr 1984), differences in $\delta^{13}C$ (the ratio $^{13}C/^{12}C$ expressed in relation to the recognized standard in parts per thousand) among animals suggest alternative pathways of carbon flow. These findings have given credence to the belief that $\delta^{13}C$ analysis can discriminate between allochthonous and autochthonous carbon sources in stream ecosystems (Rounick and Winterbourn 1986). However, the literature contains opinions ranging from enthusiastic support for (e.g., Rounick et al. 1982), to outright dismissal of (e.g., Peterson et al. 1986), stable isotope analysis (SIA) in identifying such carbon pathways. Moreover, while some studies promote precise allochthonous–autochthonous mixing models of food source origin (Junger and Planas 1992, 1994), other work seriously questions such an approach (Winterbourn et al. 1986; Rosenfeld and Roff 1992). These different opinions underscore the difficulty of reaching generalized conclusions from only a single or few investigated system(s), especially given the relatively small sample sizes employed in most stable isotope studies.

I compiled the extant $\delta^{13}C$ data on lotic foodwebs to search for general patterns from which the utility of SIA could be appraised. Specifically, I was interested in examining several of the hypotheses that have often motivated the use of carbon SIA in lotic systems.

(1) The $\delta^{13}C$ value of terrestrial detritus is uniformly and invariably −27‰, the value most often cited in the literature.

(2) Because lotic research has been biased toward the study of small, headwater streams (Minshall 1978), organisms should as a result have $\delta^{13}C$ values which approximate −27‰, demonstrating the dependence upon allochthonous material as suggested by the River Continuum Concept.

(3) Algae are often depleted in ^{13}C relative to allochthonous litter (such as found in lakes; Rau 1980), thereby enabling the tracing of alternative energy pathways; i.e., the prerequisite that "potential foods must be isotopically distinct" (Rounick and Hicks 1985).

(4) The importance of allochthonous organic matter to stream ecosystems can be assessed when riparian trees are removed by measuring the resulting changes in faunal $\delta^{13}C$ that arise from an increasing reliance upon algal carbon which proliferates when more light reaches the water.

My interest in examining the utility of SIA for tracing carbon pathways in stream ecosystems was to obtain a simple biomonitoring tool for gauging the aquatic effects of catchment deforestation. The hypothesis that faunal $\delta^{13}C$ changes predictably and quantitatively after a reduction in the supply of terrestrial litter underlies the attraction in SIA for landscape managers and environmental scientists. Unfortunately, as exemplified in the introductory quotations, it may very well be that Rosenfeld's shift from theoretical optimism (Rosenfeld and Mackay 1987) to jaded experience (Rosenfeld and Roff 1992) is an inevitable progression as one examines carbon SIA more closely.

Data

Stable carbon isotope values were obtained for 649 stream consumers, 97 lotic algal samples, and 57 submerged samples of terrestrial leaf litter directly from published tables and from figures with use of a digitizing reader. Data sources are as follows: Rau (1980); Rounick et al. (1982); LaZerte (1983); Winterbourn et al. (1984, 1986); Rounick and Hicks (1985); Araujo-Lima et al. (1986); Schell and Zieman (1988); Bunn et al. (1989); Kline et al. (1990); Rosenfeld and Roff (1992); Schallenberg (1992); Junger and Planas (1993, 1994); Mihuc and Toetz (1994); Winterbourn and Rounick (1994) in addition to those listed in France (1995*a*, 1995*b*, and 1995*c*).

Examination of hypotheses

1. Allochthonous litter approximates −27‰ $\delta^{13}C$

Because terrestrial foliage is often identified as having an average $\delta^{13}C$ value between −26‰ (Fry and Sherr 1984) and −28‰ (Peterson and Fry 1987), several lotic studies have automatically assumed allochthonous litter to range in $\delta^{13}C$ from −25‰ (Rounick and Winterbourn 1985) to −27‰ (Winterbourn et al. 1984, 1985; Winterbourn and Rounick 1985) without directly measuring the actual values. Terrestrial foliage, however, can vary considerably in $\delta^{13}C$ in relation to species, season, climate, and habitat (e.g., Garten and Taylor 1992). Other work has indicated that ^{13}C in plant tissue becomes progressively depleted during decomposition (Benner et al. 1987; Fogel et al. 1989). Despite these effects, the $\delta^{13}C$ values of submerged terrestrial litter collected during its early-to-mid stages of decomposition, shows a marked consistency (Fig. 1*a*). Until more detailed data are available on the role of heterotrophic conditioning on $\delta^{13}C$ (important because δ-^{15}N analysis suggests that invertebrates may assimilate microbial biofilms rather than leaf substrates; R. France, unpublished data), it is reasonable to assume that a large portion of the supply of allochthonous organic matter (i.e., leaves) to streams can be identified as having a $\delta^{13}C$ value of −28‰, close to that generally assumed.

Fig. 1. Percentage frequency distributions of stable isotope ratios for submerged terrestrial leaf litter (allochthonous sources) and attached algae (autochthonous sources) available as the carbon food base of streams.

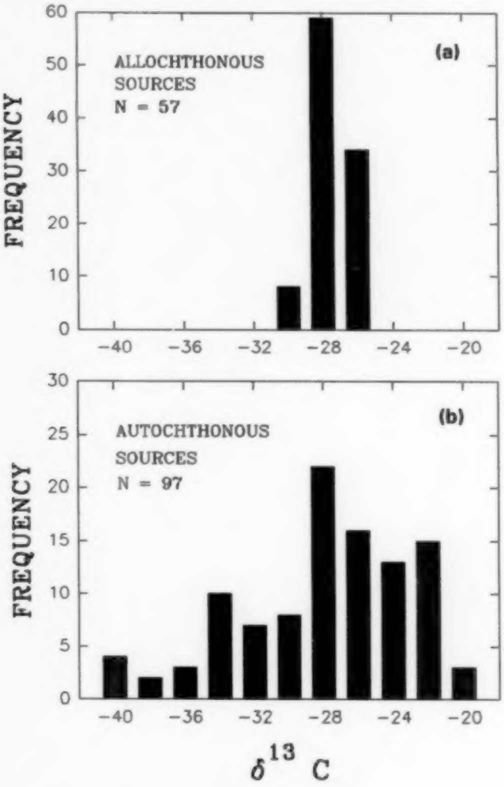
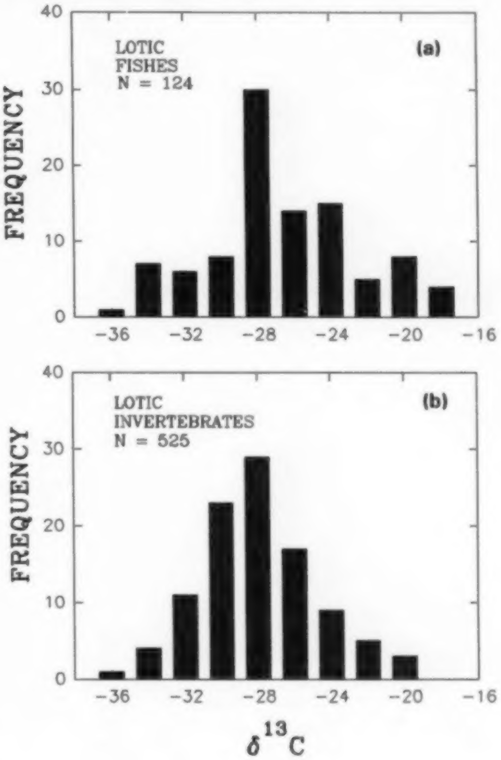


Fig. 2. Percentage frequency distributions of stable carbon isotopes for consumers in streams.



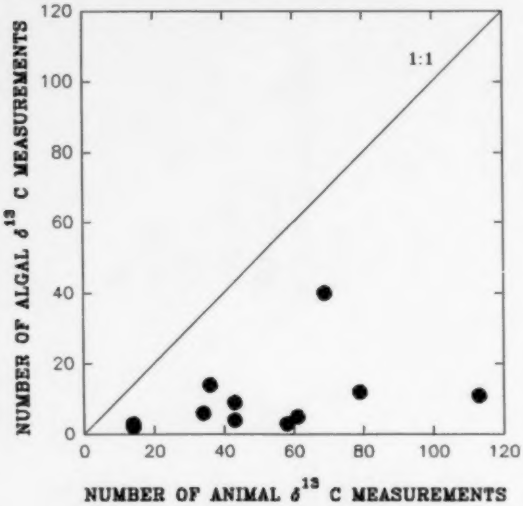
2. Lotic animals approximate −27‰ $\delta^{13}C$

Stream fishes and invertebrates exhibit unimodal frequency distributions of $\delta^{13}C$ centred about respective means of −27 and −28‰ (Fig. 2). Half of the fishes and 70% of the invertebrates had $\delta^{13}C$ values within the range encompased by terrestrial litter.

These results, mainly from headwater streams, superficially indicate that the following statements may be generally valid (albeit possibly for the wrong reasons, as discussed below): "The $\delta^{13}C$ values for…invertebrates [−23.9 to −29.5‰], indicated a clear dependence on terrestrial vegetation as food" (Rounick et al. 1982); "Most benthic invertebrates…had $\delta^{13}C$ values close to those of fine particles [−26.6 to −27.9‰], indicating that they were using carbon sources predominantly of terrestrial origin" (Winterbourn et al. 1984); "Stable isotope ratios of fine particulate organic matter…ranged from −27.4 to −28.4‰ indicating it was primarily of terrestrial origin. Most animals…had comparable values…and it can be inferred that terrestrial plant material was their main source of carbon" (Winterbourn and Rounick 1985); "Animals…had $\delta^{13}C$ values of approximately −27‰ indicating that food resources utilized were mainly of terrestrial origin," "$\delta^{13}C$ values of −24 to −28‰ obtained for animals…indicate that they were primarily dependent on terrestrial carbon resources," "All species had values slightly greater than −28.1‰…[which] indicate a reliance on materials of terrestrial origin as food" (Winterbourn et al. 1986).

Minshall (1978) believed that the idea of a general dependency of streams on allochthonous organic materials (such as suggested by the mean faunal $\delta^{13}C$ value of about −28‰ from Fig. 2) had been overestimated and that rather "in many cases autotrophy is of primary importance". Even though most of the data in Fig. 2 come from small, headwater streams located within deciduous forested catchments, Minshall's belief seems justified. Fully one-half of the fishes and almost one-third of all invertebrates displayed $\delta^{13}C$ values outside the range that can possibly be ascribed to allochthonous sources (i.e., −26 to −30‰). Therefore, autotrophic pathways within forested headwaters are clearly much more important to lotic foodwebs than would be indicated by their particulate inputs alone. Although a longitudinal continuum in relative allochthony–autochthony may exist, it would be erroneous to simply characterize all headwater streams as being "heterotrophic" and all higher order streams as being "autotrophic." Consequently, statements such as "excessive C-13 enrichment found in fish (i.e., $\delta^{13}C$ values above −27‰) must be caused by an unrecognized carbon source, *probably of terrestrial origin*" (Rounick and Hicks 1985; italics mine) reflect a

Fig. 3. Comparisons of the sampling effort used to characterize animal $\delta^{13}C$ and that used to identify algal source $\delta^{13}C$ in studies that measured both.



paradigmatic misconception in the exclusive dependence of headwater streams on allochthony.

3. Algal $\delta^{13}C$ is distinct from that of allochthonous litter

Lotic algae exhibited an enormous 20‰ range in $\delta^{13}C$ which completely enveloped that existing for terrestrial litter (Fig. 1). The mean (±SD) value for autochthonous carbon (−29 ± 4 ‰) was not significantly different from that for allochthonous carbon (−28 ± 1‰). Indeed, almost half of the $\delta^{13}C$ values determined for lotic algae are actually identical to those measured for terrestrial leaf litter.

As Bunn et al. (1989) stated, "the utility of stable isotope tracing of energy flow in ecosystems depends on the existence of differences in isotope ratios among potential food sources of energy available to consumers." This being so, the potential of SIA for tracing carbon pathways in stream ecosystems is very low (Fig. 1). For example, for those 50% of all the fishes and 70% of all the invertebrates that had $\delta^{13}C$ values between −30 and −26‰ (Fig. 2), it is completely impossible to determine the extent of dependency on allochthonous or autochthonous carbon. Therefore, as a general observation, a $\delta^{13}C$ value of about −28‰ for stream consumers, despite the beliefs outlined in the aforementioned quotations, does *not* imply a dependency upon allochthonous carbon.

The success of SIA in tracing carbon pathways in stream ecosystems appears to be very much stream specific. Some studies indicate that $\delta^{13}C$ values of allochthonous sources (leaf litter = −28‰) are distinct from those of autochthonous sources (attached algae = −35‰, Rounick et al. 1982; −34‰, Winterbourn et al. 1988; −22‰, Bunn et al. 1989; −26‰, Junger and Planas 1992; and −22 to −24‰, Junger and Planas 1994), whereas other research shows this separation to be difficult (Winterbourn et al. 1986; Rosenfeld and Roff 1992) or impossible (Peterson et al. 1986; Junger and Planas 1994) to make in some situations.

The great limitation of SIA in separating allochthonous–autochthonous dependency in streams arises from its "coarse resolving power" (Rounick and Hicks 1985) in relation to the notable variability that exists in algal $\delta^{13}C$ both within and among sampling sites (Winterbourn et al. 1986; Rosenfeld and Roff 1992). The first detailed investigation of $\delta^{13}C$ in streams (Rounick et al. 1982) was justified by the fact that because ^{13}C is depleted in lentic phytoplankton compared with terrestrial litter (e.g., Rau 1980), a similar relationship was to be expected between lotic attached algae and terrestrial litter. It is now clear that such an expectation is not realized; i.e., proportionally just as many lotic algal samples are as likely to be enriched as they are to be depleted in ^{13}C, relative to those of terrestrial litter (Fig. 1). This occurs because lotic attached algae behave quite differently from lentic planktonic algae in discriminating between carbon isotopes during photosynthesis. The great turbulence to which phytoplankton are exposed reduces the boundary layers around their cells, thereby allowing a greater discrimination against ^{13}C (France 1995*a*). This depletes the amount of ^{13}C in lake phytoplankton compared with that of lotic attached algae. The $\delta^{13}C$ values of lentic phytoplankton are therefore more negative and also more distant from terrestrial $\delta^{13}C$ values than are those of lotic periphyton.

Carbon isotope ratios of aquatic plants are regulated by variable conditions of water turbulence (e.g., Osmond et al. 1981), illumination (e.g., Wiencke and Fischer 1990), and temperature (e.g., Sackett et al. 1965). Because all these environmental factors can be quite variable even within reaches of the same stream, it should be no surprise that algae vary greatly in $\delta^{13}C$ (Fig. 1). For this reason, Rosenfeld and Roff (1992) were correct in their understanding that because algal $\delta^{13}C$ is extremely site specific, no single sample will represent the autotrophic carbon base within any single stream (or even single reach). Unfortunately, the sampling effort that has been employed to characterize consumer $\delta^{13}C$ is very large relative to that used to identify autochthonous sources (Fig. 3). As a result, the variability in algal $\delta^{13}C$ is rarely appreciated. Some work has even considered that the $\delta^{13}C$ of attached algae "is *expected* to be about −33‰" (Rounick et al. 1982; italics mine) without actually measuring it directly. In reality, only Kline et al. (1990) have come close to indicating the true variability in algal $\delta^{13}C$ within a particular stream system (in their case, a range of 17‰). Even greater variability probably exists in the other systems that have been studied but has not yet been detected because of inadequate sampling. As Winterbourn et al. (1986) concluded "aquatic ecologists must be cautious in their interpretation of absolute $\delta^{13}C$ values because of this variability and be especially careful in extrapolating from one situation to another." The present inability to confidently predict algal $\delta^{13}C$ is the greatest limitation in the use of SIA for discerning carbon pathways in stream ecosystems.

4. Riparian deforestation will cause predictable changes in stream faunal $\delta^{13}C$

Early work suggested that animals are generally more depleted in ^{13}C at open sites compared with forested sites presumably because of a greater use of autochthonous materials (Rounick et al. 1982; Rounick and Hicks 1985; Winterbourn et al. 1986). However, Fig. 1 implies that if allochthonous litter is removed as a food source, animals that shift to algae for sustenance could either decrease, increase, or remain unchanged in their $\delta^{13}C$ values. Indeed, this was found to be the case when data were examined for differences in $\delta^{13}C$ between forested and open stream sites (Fig. 4). In almost 80% of the cases, there was little (i.e., ± 3‰) or no change in animal $\delta^{13}C$ with landscape modification. Again, this result is to be expected given that the majority of both attached algae and animals have $\delta^{13}C$ values within the range −28 ± 3‰ (Figs. 1, 2).

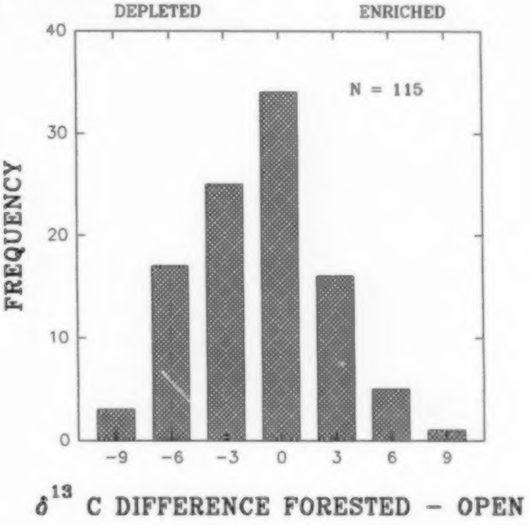
The inadequate identification of autochthonous $\delta^{13}C$ sources and possible confounding by environmental co-determinants severely limits conclusions about the effect of riparian tree removal on stream carbon isotope ratios. For example, Rounick et al. (1982) interpreted the depletion they observed in ^{13}C for invertebrates at an open site relative to a forested site as evidence for a food shift away from allochthony. However, it is entirely possible that this difference is due to the fact that the stream at the open site had a discharge over 30 times higher than that of the stream at the closed site. In such a situation, greater water turbulence would be expected to deplete algal ^{13}C (France 1995*a*) and consequently faunal ^{13}C as well. Therefore, rather than being an environmental biomonitor quantifying the effects of catchment deforestation, animal $\delta^{13}C$ in this example may simply be measuring the influence of hydrodynamics on carbon food sources. Until more is known about how algal $\delta^{13}C$ in streams responds to alterations in current, light, and temperature, all of which vary following catchment clearcutting, it will be futile to formulate recommendations for landscape managers based on SIA. The utility of SIA in understanding anthropogenic alterations to the carbon flow of stream ecosystems is presently minimal.

Discussion

From this secondary analysis of literature data, three general conclusions can be reached regarding the use of carbon SIA as a means for tracing carbon pathways in stream ecosystems. First, forested headwater streams most certainly do rely to some degree upon autotrophy in addition to allotrophy. Second, because of the great variability in attached algal $\delta^{13}C$, it may be difficult at best or even outright impossible at times to distinguish between ultimate food source origins. Finally, as a result of this, the utility of carbon SIA as a biomonitor of the lotic effects of riparian deforestation presently remains minimal.

The purpose of my secondary analysis is to caution researchers against ascribing too much faith to simple proportional mixing models of allochthony–autochthony based on carbon SIA in streams. There are, however, certain situations where carbon SIA might still have some merit. (*i*) It is possible that within small managed river sections or experimental flumes that the autotrophic carbon signatures might be both more consistent and more distinct from those of allochthonous material than suggested through the present compilation of data originating from many different regions. (*ii*) As Peterson and Fry (1987) and others have noted, it is likely that the most significant advances in stable isotope research will be made with the simultaneous use of more than one isotope. Secondary analysis of literature data (France 1995*b*) indicates that terrestrial plants and aquatic algae–plants can often differ in their respective $\delta^{15}N$ signatures, thereby raising the possibility that some combination of both carbon and nitrogen SIA may be a profitable direction worth pursuing further. (*iii*) Because of increased boundary layer diffusion resistance, the $\delta^{13}C$ values of attached algae in stagnant waters such as lake littoral zones have a much greater likelihood of being distinct from those of terrestrial plants (France 1995). If stream ecologists are interested in discriminating allochthonous and autochthonous carbon pathways with carbon SIA, the most suitable river locations in which to undertake such research will therefore be in pools characterized by only minimal water movement.

Fig. 4. Frequency distribution of differences in stable carbon isotopes between the same species sampled in forested and in open streams. Negative values indicate organisms at open sites are depleted in ^{13}C relative to those at forested sites, whereas positive values indicate organisms at open sites are enriched in ^{13}C relative to those at forested sites.



There are at least two subject areas that require considerable research before carbon SIA can enjoy widespread and scientifically defensible use in investigations of carbon flow in streams. (*i*) Paramount is the need to obtain much more detailed information about the environmental determinants regulating the $\delta^{13}C$ values of attached algae from which general predictions within the Continuum framework might be made. Such work would be most valuable in concordance with pigment analysis of the attached algal community to discern true autochthonous carbon sources from entrapped allochthonous detritus. (*ii*) Multiple isotopic analysis of the particles associated with leaf breakdown would be greatly beneficial toward identifying precise food sources for macroinvertebrates. By knowing what proportion of the algal biofilm, bacterial biofilm, or actual leaf substrate itself is consumed may help to explain the resulting $\delta^{13}C$ signatures of higher trophic organisms.

In summary, although as currently practiced, SIA of stream carbon pathways raises more questions than it answers.

Future attention devoted to many of the confounding variables and potentially profitable research directions addressed in this review would go far toward ameliorating some of the progressive skepticism displayed in the introductory quotations as one examines this research tool more closely.

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Les gouvernements du Canada et du Québec et la Ville de Québec sont heureux de vous inviter au Symposium International du 50e anniversaire de l'organisation des Nations Unies pour l'alimentation et l'agriculture (FAO) à Québec, Canada. Il portera le thème **"L'humain au coeur du développement : la sécurité alimentaire par son savoir-faire".**

11-13 octobre 1995

L'esprit du Symposium

- Mettre l'accent sur la sécurité alimentaire des peuples.
- Privilégier le savoir-faire comme piste principale dans la recherche de solutions.
- Inviter des personnalités politiques et des acteurs «terrain» (entreprises et ONG) déterminés à s'engager dans la recherche de solutions originales.

Trois sous-thèmes

- La gestion des ressources naturelles.
- La gestion des marchés.
- La gestion des savoirs et de la technologie.

Information ou inscription
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Symposium FAO 1995
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Three Sub-Themes

- Managing Natural Resources.
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- Managing Know-how and Technology.

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ANNOUNCEMENTS / ANNONCES

15th International Symposium of the North American Lake Management Society (NALMS)

Toronto, Ontario
November 6 - 11, 1995

The 15th International Symposium of NALMS will be held in Toronto, Ontario, Canada, at the Westin Harbour Castle, November 6-11, 1995. Our theme is *Aquatic Ecosystem Stewardship*. The purpose of NALMS is "to forge partnerships among citizens, scientists, and professionals to foster the management and protection of lakes and reservoirs for today and tomorrow". The Federation of Ontario Cottagers Association (FOCA) has agreed to join us in Toronto for their annual autumn meeting. FOCA has an active environment committee and a strong Lake Steward programme.

Special sessions include recovery of damaged ecosystems, lake management north of 60, preimpoundment planning of reservoir management, fish stocking as a management tool, rehabilitation and management of urban lakes and reservoirs, anoxia and phosphorus, and wetland restoration.

For further information contact

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telephone: 705-766-2418
fax: 705-766-2254

15th International Symposium of the North American Lake Management Society (NALMS)

Toronto, Ontario
du 6 au 11 novembre 1995

Le 15e symposium international de la NALMS aura lieu à Toronto (Ontario), Canada (au Westin Harbour Castle), du 6 au 11 novembre 1995. Le thème choisi est La gestion des écosystème aquatiques. Le but de NALMS est de "créer des partenariates entre les citoyens, les scientifiques et les professionnels pour encourager l'aménagement et la protection des lacs et des réservoirs pour le présent et l'avenir". La fédération ontarienne des propriétaires de chalets (FOCA) a décidé de se joindre à nous à Toronto pour tenir leur réunion annuelle de l'automne. FOCA comprend un comité environnemental très actif et un programme de Gérants des Lacs.

Quelques sessions planifiées sont la réhabilitation des écosystèmes endommagés, l'aménagement des lacs au nord du 60ième parallèle, la planification des réservoirs avant inondation, la restauration et l'aménagement des lacs urbains, l'ensemencement des lacs comme outil de gestion, l'anoxie et le phosphore, et la restauration des marécages lacustres.

Pour de plus amples renseignements, communiquez avec

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Contents (concluded) / Table des matières (suite et fin)

Haakon Hop, Mark Graham, and Vance L. Trudeau 541–550 Spawning energetics of Arctic cod (*Boreogadus saida*) in relation to seasonal development of the ovary and plasma sex steroid levels

Peter Blomqvist, Russell T. Bell, Hans Olofsson, Ulrika Stensdotter, and Katarina Vrede 551–565 Plankton and water chemistry in Lake Njupfatet before and after liming

Larry D. Jacobson and Alec D. MacCall 566–577 Stock-recruitment models for Pacific sardine (*Sardinops sagax*)

J.D. Fitzsimons, J.H. Leach, S.J. Nepszy, and V.W. Cairns 578–586 Impacts of zebra mussel on walleye (*Stizostedion vitreum*) reproduction in western Lake Erie

Dale H. Vitt, Suzanne E. Bayley, and Tai-Long Jin 587–606 Seasonal variation in water chemistry over a bog-rich fen gradient in Continental Western Canada

L.A. Cocchio, D.W. Rodgers, and F.W.H. Beamish 607–613 Effects of water chemistry and temperature on radiocesium dynamics in rainbow trout, *Oncorhynchus mykiss*

M.C. Healey and U. Reinhardt 614–622 Predator avoidance in naive and experienced juvenile chinook and coho salmon

Karen Young Kreeger 623–630 Differences in the onset of salinity tolerance between juvenile chinook salmon from two coastal Oregon river systems

R.G. Randall, J.R.M. Kelso, and C.K. Minns 631–643 Fish production in freshwaters: Are rivers more productive than lakes?

Richard J. Beamish and Chrys-Ellen M. Neville 644–650 Pacific salmon and Pacific herring mortalities in the Fraser River plume caused by river lamprey (*Lampetra ayresi*)

REVIEW / SYNTHÈSE

Robert France 651–656 Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems

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Volume 52, Number 3, March 1995

Volume 52, numéro 3, mars 1995

Laura A. Bankey, Peter A. Van Veld, Dennis L. Borton, Larry LaFleur, and John J. Stegeman 439–447 Responses of cytochrome P4501A in freshwater fish exposed to bleached kraft mill effluent in experimental stream channels

Ian A. McLaren and Patricia Avendaño 448–463 Prey field and diet of larval cod on Western Bank, Scotian Shelf

Todd M. Koel and John J. Peterka 464–469 Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota

Anthony Ricciardi, Robert Serrouya, and Frederick G. Whoriskey 470–477 Aerial exposure tolerance of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal

M.O. Hammill, M.C.S. Kingsley, G.G. Beck, and T.G. Smith 478–488 Growth and condition in the Northwest Atlantic harp seal

D.W. Welch, A.I. Chigirinsky, and Y. Ishida 489–503 Upper thermal limits on the oceanic distribution of Pacific salmon (*Oncorhynchus* spp.) in the spring

A.R. Appenzeller and W.C. Leggett 504–511 An evaluation of light-mediated vertical migration of fish based on hydroacoustic analysis of the diel vertical movements of rainbow smelt (*Osmerus mordax*)

Aristocle Ndayibagira, Marie-Josée Cloutier, Perry D. Anderson, and Philip A. Spear 512–520 Effects of 3,3′,4,4′-tetrachlorobiphenyl on the dynamics of vitamin A in brook trout (*Salvelinus fontinalis*) and intestinal retinoid concentrations in lake sturgeon (*Acipenser fulvescens*)

W.L. Silvert and A.D. Cembella 521–531 Dynamic modelling of phycotoxin kinetics in the blue mussel, *Mytilus edulis*, with implications for other marine invertebrates

Miki Ogura and Yukimasa Ishida 532–540 Homing behavior and vertical movements of four species of Pacific salmon (*Oncorhynchus* spp.) in the central Bering Sea

Continued on inside back cover / Suite au verso

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